THE EFFECT OF MURISTERONE (A PHYTOECDYSON) ON GROWTH, MOULTING, METAMORPHOSIS AND REPRODUCTION OF THE RED COTTON BUG, *Dysdercus cingulatus* (HEM : PYRRHOCOREIDAE)

M. Phil. Dissertation

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CERTIFICATE

This is to certify that the research work presented in the dissertation entitled "The effect of Muristerone (a phytoecdyson) on growth, moulting, metamorphosis and reproduction of the red cotton bug, Dysdercus cingulatus (Hem:Pyrrhocoridae) by Mr. Bachchan Ali Khan is original and was carried out under my supervision. He is permitted to submit it for the partial fulfilment of the degree of Master of Philosophy in Zoology, at Aligarh Muslim University, Aligarh, India.

Mumtaz Ahmad Khan

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C O N T E N T S

A C K N O W L E D G E M E N T S

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(BAGHCHAN ALI KHAN)

I. INTRODUCTION

In insects moulting hormone or ecdyson is produced and secreted by the prothoracic glands of the larvae of many insects and the ring glands of the larvae of cyclorrhaphous Diptera. But the secretion of this hormone is stimulated by prothoracicotropic hormone or neurosecretory material produced by the median neurosecretory cells of the brain (Wigglesworth, 1973; Hyatt, 1971; Doane, 1973; Gilbert and King, 1973). It is well known that the larval growth and moulting in insects is regulated by the co-ordination of juvenile hormone (liberated from the corpus allatum) and moulting hormone. Ecdyson or moulting hormone provokes a developmental response when it gets accumulated in threshold titre at the critical periods. Whereas, titre of juvenile hormone determines whether the developmental response will be moulting or metamorphosis.

The prothoracic glands produce ecdyson in α- and β-forms but following liberation α-form also becomes β-isomer. However, the ecdysones are steroids in nature. Apart from the biological significance of the endogenous ecdysones, the effects of exogenous ecdysones have also been studied when applied on various stages of development. A number of

analogues of β-ecdyson have been synthesized. Further, such derivatives have also been isolated from plants especially ferns and gymnosperms and resemble ecdyson in biological activity. Thus these hormones are called 'phytoecdysones' or 'phytoecdysteroids'. The moulting hormone and its related compounds are nonspecific in action. The application of exogenous ecdysones and their analogues including phytoecdysones result in moulting disorders (Earle et al., 1970; Robbins et al., 1970; Thompson, 1970; Martelli, 1978; Singh and Russell, 1980 and 1982; Ahmad and Khan, 1982 and 1985; Kubo et al., 1983), suppression of metamorphosis (Bowers, 1968; Robbins et al., 1968) and cause inhibitory effects on reproduction (Robbins et al., 1968; Earle et al., 1970, Kaplanis et al., 1970; Ahmad and Khan, 1982 and 1985; Khan et al., 1984). However, in some species these hormones in sublethal doses stimulated the growth and reproduction (Laverdure, 1975; Herman and Barker, 1976; Went, 1968; Chudakova et al., 1982).

Since exogenous ecdysones are generally nontoxic to the non-target organisms, these may become a possible alternative to other methods or may a part of integrated method of insect control. It is therefore, important to evaluate the effect of ecdyson analogue as well as that of phyto-

ecdysones on a variety of insect pests in the laboratory. The present programme deals with a part of Scheme of investigation on several insect pests. The present insect is red cotton bug, Dysdercus cingulatus which is a cosmopolitan pest of cotton and other malvaceous plants including certain vegetables in India. Sub-lethal doses of a phytoecdyson Muristerone were applied on the advanced nymphal stages of this species and then growth, moulting, metamorphosis, fecundity, and fertility were studied not only in the present generation but also in F_1 and F_2 generations for any residual effect of this hormone.

II. REVIEW OF LITERATURE

Effects of moulting hormones (ecdysones) and their various analogues have been studied on a number of biological activities in several insect species. However, the present review deals with the major observations on the effects of exogenous ecdysones including synthetic analogues as well as phytoecdysones on the growth and reproduction of insects.

THYSANURA

Robbins et al. (1968) observed the inhibition of growth, moulting, metamorphosis and reproduction in Thermobia domestica following the injection of α-ecdyson, β-ecdyson and certain synthetic analogues.

ORTHOPTERA

α-ecdyson: Injection of ecdyson (α-ecdyson) into the immature females of Locusta migratoria resulted in hastening the oocyte growth for 24 hours which later degenerated (Joly et al., 1968).

β-ecdyson: Injection of β-ecdyson (0.5 to 6.0 µg/nymph) into the 4th and 5th instar nymphs of *H. roglyphus nigrorepletus* caused larval mortality, moulting inhibition (Khan et al., 1984). In *Schistocerca gregaria* metamorphosis was delayed when 24 and 48 hours old nymphs were injected 20-hydroxyecdyson (β-ecdyson). On the contrary it was advanced when 72 hours old nymphs were treated with the same hormone (El-Ibrashy et al., 1976). Khan et al., (1984) further observed that β-ecdyson injection into the 4th and 5th instar nymphs of *H. nigrorepletus* caused reduction in the fecundity and fertility. Masnier and Thomas (1982) in *Carausius morosus* observed that an overdose of β-ecdyson caused disturbances of follicular cell activity involving malformation of capitulum of the eggs. Whereas, Maslinikova and Chudakova (1982) reported that injection of 20-hydroxy-ecdyson into the newly emerged females of *Acheta domestica* stimulated development of oocytes at a dose of 1 µg/insect but inhibited it at 5 or 10 µg/insect.

Makisterone A and Triol: Khan et al. (1984) further observed that injection of Makisterone (a phytoecdyson) and Triol (an ecdyson analogue) at doses 0.5 to 6.0 µg/hopper into the 4th and 5th instar nymphs of *H. nigrorepletus* caused nymphal mortality, ecdysis inhibition and reduction in the fecundity of females. Triol also reduced the egg fertility.

DICTYOPTERA

α- ecdyson: In *Leucophaea madrae* inhibition of oocyte growth was observed by Earle et al., (1959) following the injection of natural ecdyson (α-ecdyson). Further, Robbins et al., (1968 & 1970) and Thompson (1971) reported that ingestion of α-ecdyson by the nymphs of *Blatella germanica* inhibited nymphal development, growth and reproduction.

β-ecdyson: Growth, development and reproduction were inhibited following the ingestion of β-ecdyson by *Blatella germanica* nymphs (Robbins et al., 1968 & 1970); Friedal et al., 1980).

HEMIPTERA

α-ecdyson: According to Ahmad (1983) injection of α-ecdyson (0.5 to 6.0 µg/nymph) into the 4th and 5th instar nymphs of *Dysdercus cingulatus* caused nymphal mortality, ecdysis inhibition and shortening of nymphal longevity as well as reduction in the fecundity and fertility. Injection of α-ecdyson into the adults of *Oncopeltus fasciatus* resulted in early appearance of vitellogenin in the haemolymph (Rankin and Jakle, 1980). Whereas, Garcia et al., (1979) found the dose dependent inhibition of oviposition and oogenesis following the ingestion of ecdyson (α-ecdyson) by *Rhodnius prolixus*.

B-ecdyson: Jalaja et al. (1976) in D. cingulatus and Mansingh (1976) in R. prolixus separately observed that injection of B-ecdyson caused mortality and inhibition of vitellogenesis and oviposition. Further, Rankin and Jakle (1980) reported the early appearance of vitellogenin in the haemolymph of Oncopeltus fasciatus following the injection of 20-hydroxyecdyson.

Triol: Injection and topical application of Triol (an ecdyson analogue in 0.5 to 6.0 µg/nymph) to the 4th and 5th instar nymphs of D. cingulatus caused nymphal mortality moulting inhibition, shortening of nymphal period as well as drop in the fecundity and fertility (Ahmad and Khan, 1985).

LEPIDOPTERA

α-ecdyson: Williams (1968) found that α-ecdyson injected into the diapausing pupae or isolated pupal abdomens of silkworm, Samia cynthia provoked normal or abnormal development depending on the dose. According to Calvez (1976) in Bombyx mori and Hwang Hsu (1979) in Galleria sp. α-ecdyson injection to the first day of the last larval instar induced synthesis of larval cuticle. However, if the treatment was made later, the new cuticle was pupal instead

of larval. Acceleration of gut-purge by 6.5 hours in S. cynthia was observed following the injection of α-ecdyson (Fujushita et al., 1982). Ahmad (1983) observed that α-ecdyson injection into the larvae of Dicrisia obliqua caused larval mortality, moulting inhibition as well as larval, pupal and adult malformation. Novock (1972) reported that α-ecdyson injection (0.5 µg/insect) induced fusion and torsion in the testes of pharate pupae of Ephestia kuhniella. Further, according to kambysellis and Williams (1977) injection of α-ecdyson into the pupae of S. cynthia accelerated the spermatogenesis. Nijhout (1976) applied α-ecdyson topically on the last instar larvae of Manduca sexta and found that treatment of one day old larvae induced the synthesis of larval cuticle, whereas later treatment resulted in the induction of pupal cuticle instead of larval. Inhibition of development of B. mori larvae was found by Martelli (1978) following the ingestion of α-ecdyson. Ahmad (1983) observed fall in the fecundity and fertility in D. obliqua females as a result of α-ecdyson ingestion to the 5th and 6th instar larvae. Spermatogenesis was accelerated in Mamestra brassicae following in-vitro application of α-ecdyson (Fukushima and Yagi, 1975).

β-ecdyson: Injection of ecdysterone (β-ecdyson) into the last instar larvae of Corcyra cephalonica (Raghwan and Nadkarni, 1977) and B. mori (Anonymous, 1979) caused early formation of puparium. Hamada (1984) found that injection of 20-hydroxyecdyson (β-ecdyson) into the two days old female pupae of B. mori retarded adult emergence up to 8 days. Kobayashi and Burdette (1962) in B. mori and Giebultowiz et al., (1980) in Ephestia kuhniella reported that injection of β-ecdyson caused failure of moulting cycle, formation of larval-pupal and pupal-adult intermediates and consequently death. Formation of supernumerary instar as well as malformation of larvae, pupae and adults occurred in S. litura as a result of injection of β-ecdyson into the 5th and 6th instar larvae (Khan et al., 1984). Beck and Shane (1969) observed induction of apolysis in diapausing larvae of Ostrinia nubilalis following the injection of a high dose of β-ecdyson. Injection of β-ecdyson terminated diapause and induced normal or abnormal development of the pupae of S. cynthia (Williams, 1968), Monema flavescens (Takeda, 1978; Brad field and Denlinger, 1980), Laspeyresia pomonella (Sieber and Benz, 1980) and M. configurata (Boknaryk, 1985). Khan et al., (1984) found that injection of β-ecdyson into the 5th and 6th instar larvae of S. litura reduced fecundity and fertility of the females. Similar

treatment of the monarch butterfly at low doses (0.01 to 1.0 µg/female) inhibited ovarian development but higher doses (10 µg/female) stimulated both male and female reproductive glands (Herman and Barker, 1970). Further, Chatani and Ohnishi (1976) found that injection of this hormone into isolated pupal abdomens of B. mori induced initiation of ovarian development. According to Thorson and Rieman (1982) injection of aquous 20-hydroxyecdyson into adult males of Anagasta kuhniella prevented the impending release of eupyrene sperm-bundles. But the release of apyrene sperm-bundles was not prevented.

Sato et al. (1968) observed the induction of growth and development following the topical application of β-ecdyson on the isolated larval abdomens of Chilo suppressalis and Plutella xylostella. Similarly, topical application of this hormone on the larvae and pupae of B. mori accelerated the development (Hagesawa and At/ 1971 & 1972). Further, Kiruma (1974) observed that similar application on the diapausing pupae and eggs of this insect induced the development. Similarly, topical application of β-ecdyson on the isolated larval abdomens of M. sexta also induced the development (Nijhout, 1976; Riddiford and Curtis, 1978). Ovicidal action of β-ecdyson was reported by Ayad and Bishare (1981) on S. littoralis eggs.

β-ecdyson accelerated the growth of B. mori when given in the diet of the 4th instar larvae but the same treatment to the 5th instar larvae caused inhibition in growth (Shigamatsu et al., 1974). According to Martelli (1978) in B. mori and Kubo et al., (1981) in B. mori and Pectinophora gossypiella, ingestion of β-ecdyson by the larvae caused failure of moulting cycle, formation of larval-pupal and pupal-adult intermediates and consequently death. Further, ingestion of β-ecdyson by the larvae of S. litura (Ahmad and Khan, 1982) and B. mori (Kubo et al., 1983) caused larval mortality and inhibition of ecdysis. Ahmad and Khan (1982) and Khan et al. (1984) also observed formation of supernumerary instar, malformation in the larvae, pupae, and adults, shortening of larval longevity as well as reduction in the fecundity and fertility of the females following β-ecdyson ingestion by the 5th and 6th instar larvae of S. litura . Yagi et al. (1969) found that in-vitro treatment of ecdysterone caused rapid development of testis and spermatocytes in Chilo suppressalis.

Phytoecdysones:

(a) Cyasterone:- Injection of Cyasterone (0.2 µg/pupa) into the diapausing pupae of S. cynthia caused abnormal moulting (Martelli, 1978). This hormone was ovidal when

topically applied on the eggs of S. littoralis (Ayad and Dishare, 1981). Whereas, Anonymous (1979) and Kubo et al. (1981) in B. mori larvae reported abnormal moulting following the ingestion of Cyasterone.

(b) Inokosterone: According to Kambysalis and Williams (1967) injection of Inokosterone (0.1 to 10.0 µg/pupa) into the pupae of B. mori resulted in early emergence as well as formation of pupal-adult mixtures depending on the dose. Similarly, Williams (1968) observed that injection of lower dose of this hormone into the diapausing pupae of S. cynthia provoked normal development but excessive doses and caused abnormal development when injected into the pupae or isolated pupal abdomens. Topical application of Inokosterone on the pupae of B. mori accelerated the pupal-adult development, but resulted in the formation of pupal-adult mixtures as well as adults with morphological defects (Kobayashi and Burdette, 1962; Hagesawa and At 1971 & 1972). Abnormal moulting was reported following the topical application of Inokosterone into the 3rd and 4th instar ligated larvae of Chilo suppressalis and Plutella xylostella (Sato et al., 1968). Further, Shigamatsu et al. (1974) in B. mori found inhibition of larval growth following the ingestion of Inokosterone.

Makisterone A and Muristerone: Injection of Makisterone A into the larvae of S. litura caused larval mortality, moulting inhibition as well as reduction in the fecundity and fertility (Khan et al., 1984). According to Martelli (1973) ingestion of Makisterone A and Muristerone caused inhibition of growth in S. cynthia larvae. It was also found that Muristerone was more effective in preventing the completion of development.

Ponasterone A, Ponasterone B and Ponasterone C: Injection of Ponasterone A into the ligated larvae of Chilo suppressalis and Cadra cautella caused abdominal moulting (Sato et al., 1968). However, in the diapausing pupae of S. cynthia, Williams (1968) reported that injection of critical doses of Ponasterone A, Ponasterone B and Ponasterone C provoked normal development but excessive doses caused extremely abnormal moulting. Injected Ponasterone A in B. mori larvae caused death without moulting, following promoted moulting and during promoted moulting (ecdysis inhibition) as well as inhibition of growth with and without effect on moulting (Kubo etal. 1983). On the other hand, Hagesawa and Ati (1971 & 1972) found that topical application of Ponasterone A accelerated pupal-adult development in B. mori, however, emerged females had many morphological defects. According to Nakanishi (1969), ingestion of

Ponasterone A caused premature spinning in Cecropia larvae. However, Riddiford (1970) in Cecropia and Shigamatsu et al. (1979) in B. mori reported that ingestion of Ponasterone A by the larvae inhibited the development and decreased ecdysis. Abnormal moulting and ecdysis inhibition was also observed by Kubo et al. (1983) in B. mori larvae following the ingestion of Ponasterone A.

Triol: Triol when injected into the 5th and 6th instar larvae of S. litura caused larval mortality, moulting inhibition, formation of supernumerary instar as well as reduction in the fecundity and fertility (Khan et al., 1984). On the contrary, Robbins et al. (1968) reported that Triol, (labelled as compound IV) at a dietary concentration of 37.5 ppm was almost inactive against M. sexta larvae.

DIPTERA

α-ecdyson and its analogues: Ohtaki et al. (1967) observed that injection of α-ecdyson provoked early puparium formation in Sarcophaga peregrina. Injection of lower doses of α-ecdyson and 5, B-hydroxyecdyson (α-ecdyson analogue) into the diapausing pupae of Sarcophaga crassipalpis caused early resumption of adult emergence whereas, higher doses were

effective in immediate termination of diapause and resumption of abnormal development (Zdarek and Denlinger, 1975). Accordin to Fuchs et al. (1970) α-ecdyson and 22-isoecdyson (analogue of α-ecdyson) did not initiate vitellogenesis in Aedes aegypti. Ittecheriah (1974) found that females of Culex tarsalis laid infertile eggs following the topical application of 22, isoecdyson. Thompson (1970) reported that ingestion of two analogues of α-ecdyson labelled as compounds VIIIa and VIIIb by the larvae of Musca domestica and A. aegypti caused inhibition in larval growth. Compound VIIIa also inhibited the ovarian development which was also inhibited following the ingestion of two α-ecdyson analogues viz., 2,25-dideoxy-α-ecdyson and dideoxyecdyson by the larvae of Calliphora (Galbraith et al., 1975). Whereas, Wright and Kaplanis (1970) observed inhibition of egg production and delay in oviposition following the ingestion of α-ecdyson by one day old females of Stomoxys calcitran .

β-ecdyson: Ohtaki et al. (1967) in S. peregrina and Thompson and Horn (1969) in Calliphora stygia observed that injection of β-ecdyson into the larvae provoked early puparium formation. According to Zdarek and Salma (1972) injection of ecdysterone (20 µg/larvae) into the 3rd instar larvae of S. crassipalpis caused precocious moulting and

formation of a supernumerary instar whereas, injection of this hormone (6 µg to 10 µg/g) into the pupae of S. argyrostoma led to hyperhormonal abnormalities affecting antennae, genitalia and bristle orientation (Gibbs, 1976). Fallon et al. (1974) in A. aegypti and Agui et al. (1977) in decapitated M. domestica reported that injection of β-ecdyson into the females induced vitellogenesis. On the contrary, inhibition of egg production was observed by Fraenkel and Hollowell (1979) in Phormia regina and S. bullata following the injection of β-ecdyson into the females of these insects. Huybrechts and Loof (1981) in S. bullata, Lea (1982) in A. aegypti and Redfern (1982) in Anopheles stephensi reported that β-ecdyson injection induced the vitellogenesis. Similar treatment of C. pipiens (Meixum et al., 1980) and A. aegypti (Lea, 1982) stimulated deposition of yolk into the developing oocytes. Ittecheriah (1974) found that topical application of β-ecdyson on C. tarsalis inhibited the development of eggs.

Gvozdev (1974) found that B-ecdyson feeding to the Drosophila melanogaster larvae inhibited growth of the established embryonic cells. Larval mortality and inhibition of development was observed following the ingestion of β-ecdyson by the larvae of M. domestica (Robbins et al., 1968; Singh and Russell, 1980 & 1982). Robbins et al. (1968) also reported inhibition of reproduction. Inhibition of egg development was reported in Stomoxys calcitran following the ingestion of β-ecdyson.

Phytoecdysones:

(a) Cyasterone: Cyasterone injection into the isolated larval abdomens of S. peregrina provoked early formation of puparium (Ohtaki et al., 1967). Whereas, complete metamorphosis of transplanted imaginal discs of D. melanogaster was observed by Postlethwait and Schneidermann (1968 & 1970).

(b) Inokosterone, Ponasterone A, Ponasterone B and Ponasterone C:

Injection of Inokosterone, Ponasterone A, Ponasterone B, and Ponasterone C into the isolated larval abdomens of S. peregrina provoked early puparium formation (Ohtaki et al., 1967). On the other hand, Ponasterone A, when ingested by the larvae of M. domestica (Robbins et al., 1968) and A. aegypti (Robbins et al., 1970) inhibited their development. Further, according to Wright and Kaplanis (1970) ingestion of Ponasterone A by S. calcitran females partially inhibited the egg production.

Triol: Topical application of Triol on M. domestica (Bowers et al., 1970) as well as ingestion of this hormone by M. domestica (Robbins et al., 1968) and A. aegypti (Robbins et al., 1970) caused inhibition of larval development. Further, Wright and Kaplanis (1970) reported inhibition of egg production when Triol was fed to the females of S. calcitran.

HYMENOPTERA

Loof et al. (1979) reported that topical application of ecdysterone on the larvae of parasitic wasp, Nasonia vitripenis resulted in termination of larval diapause.

COLEOPTERA

α-ecdyson: According to Socha and Sehnal (1972) adult emergence was hastened which took place in 90-120 hours instead of 144-168 hours when pupae of Tenebrio molitor were injected with 20 µg α-ecdyson/pupa. Ingestion of 22,25-bisdeoxyecdyson (α-ecdyson analogue) caused inhibition of larval growth as well as shortening of the pupal period in Epilachna varivestis (Walker and Thompson, 1973). Similarly, Robbins et al. (1970) found that ingestion of α-ecdyson at 0.5% concentration by the larvae of Tribolium confusum caused 28% inhibition of development. Mar[illegible]i (1978) also reported inhibition of larval development in T. molitor, Leptinotarsa decemlineata and Galurecella luteola following the ingestion of [illegible]-ecdyson. On the contrary, Laverdure (1975) reported that α-ecdyson (3 mg/ml of medium) in-vitro cultures of ovaries of T. molitor stimulated growth of the oocytes in very young ovaries but the development was abnormal.

β-ecdyson: Reproduction was inhibited in the females of *T. confusum* (Robbins et al., 1968 & 1970) in *T. molitor*, *L. decemlineata* and *G. luteola* (Martelli, 1978) following the ingestion of β-ecdyson by their larvae.

Phytoecdysones: Cyasterone, at 0.5% concentration in the larval diet caused 16% inhibition of the development in *T. confusum* whereas an equal dose of Ponasterone A caused 100% inhibition (Robbins et al., 1970). Martelli (1978) observed that ingestion of Makisterone A and Muristerone by the larvae of *T. molitor*, *L. decemlineata* and *G. luteola* inhibited the development . It was also observed that Muristerone was more effective in preventing the completion of development.

Triol: Robbins et al. (1968 & 1970) found that dietary Triol when given to the larvae of *T. confusum* caused larval mortality as well as inhibition of reproduction. A complete inhibition of egg production was observed following the ingestion (1%) of Triol by the adults of *Anthonomus grandis* (Earle et al., 1970).

III. MATERIALS AND METHODS

Breeding and Maintenance of Stock Culture:

The adults of Dysdercus cingulatus were collected from the cotton crops and maintained in the rearing glass jars measuring 20x15 cms containing 2 to 3 cms thick layer of moist and loose sand at the bottom. These jars containing the insects were kept at 30 ± 1°C and 70-80% R.H. in a B.O.D. incubator. All the stages of Dysdercus cingulatus were daily fed on soaked healthy cotton seeds. The females laid the eggs. Then the insects were transferred to fresh jars. On hatching the nymphs were daily provided with soaked cotton seeds.

In the present experiments newly moulted (one day old) nymphs of 4th and 5th instars were treated with the hormone.

Preparation of different doses of the hormone:

Five doses of Muristerone i.e., 0.5 µg, 1.0 µg, 2.0 µg, 4.0 µg and 8.0 µg were prepared. To make a stock solution (S) first of all 8 mg of Muristerone was dissolved in 2 ml acetone. Thus 2 µl of this solution (S) contained 8.0 µg

Muristerone. Then 1 ml of this solution(S) was diluted by adding equal quantity of the solvent to make S1 solution. Thus 2 µl of this solution (S1) contained 4.0 µg Muristerone. Similarly, S2 was prepared which had 20 µg Muristerone in 2 µl solution. In this way serial dilutions further gave 1.0 µg and 5.0 µg doses respectively in 2 µl of acetone solution.

Application of the Hormone:

Each dose was applied on one day old individual nymphs of 4th and 5th instar separately by topical and injection methods with the help of a Tuberculine syringe which was fitted into a microapplier. The topical application was made by applying each dose of the hormone on the prothorax of individual nymphs. Whereas, each dose was seperately injected into the body cavity through intersegmental membrane of the last abdominal segment. Similarly nymphs were treated with only 2.0 µl acetone solution to serve as parallel control. Untreated nymphs of corresponding stage and age were also maintained compare the results. Each dose was tested against 100 nymphs of 4th and 5th instars by either of these methods, in four replicates, each consisting of 25 nymphs at a time.

The nymphs treated with each dose were kept in separate rearing jars provided with fresh soaked cotton seeds daily and maintained at controlled conditions of the temperature and humidity.

Method for Observations:

Following the application of each dose of the hormone on 4th instar (24 hrs. old) or 5th instar (24 hrs. old) nymphs observations were made on growth, moulting, metamorphosis as well as longevity of the treated nymphs. The data were compared with those of acetone treated (control) and untreated.

The observations were also made on the fecundity and fertility of the females which emerged from the treated nymphs. For this purpose 6 females (affected) emerged from the treated nymphs of either instar were paired with equal number of normal males of corresponding age and stage. Each pair was kept in a separate rearing jar at the above mentioned conditions. The number of eggs laid by each female was recorded. When the insects were transferred to the fresh rearing jars, after hatching the nymphs were counted. The unhatched eggs were also counted. Similar observations

were also made in control consisting of the males and females emerged from acetone treated as well as untreated nymphs.

The observations were also made on the residual effect of the hormone in the successive (F1 and F2) generations. When eggs of the affected females hatched, 100 nymphs were isolated for further observations on the growth, moulting, mortality, metamorphosis, fecundity and fertility according to the above mentioned method. Similar observations were made in the parallel control.

Statistical Analysis:

The data were analysed statistically. Standard deviation (S.D.) was calculated by the following formula:

$$S.D. = \sqrt{\frac{\Sigma D^2}{n-1}}$$

where ΣD^2 = sum of square of the differences of Mean values.

n = number of observations

On the basis of Standard deviation (S.D.), Standard Error (S.E.) was calculated by the following formula:

$$S.E. = \frac{S.D.}{\sqrt{n}}$$

For the significant test the following formula was applied.

$$t = \sqrt{\frac{m1 - m2}{\frac{SD1}{n1} + \frac{SD2}{n2}}}$$

Where : t = Significant value

m1 = mean value of first set of observations

m2 = mean value of second set of observations

SD1 = Standard deviation of first set of observations

SD2 = Standard deviation of second set of observations

n1 = Number of observations of first set.

n2 = Number of observations of second set.

The calculated 't' was compared with the tabulated 't' (Bailey, 1959) at 5% level. If the former value is higher than the later, the data are significant otherwise insignificant. The tabulated value of 't' at 5% level is 2.447.

IV. RESULTS AND OBSERVATIONS

(a) Effect of topical application of Muristerone on the 4th instar nymphs of Dysdercus cingulatus:

Each of the doses viz; 0.5 µg, 1.0 µg, 2.0 µg, 4.0 µg, and 8.0 µg of Muristerone was topically applied on 100 nymphs of 4th instar (one day old) individually in four replicates, each consisting of 25 nymphs. An equal number of nymphs was treated with acetone (solvent) only to serve as control. Similarly, a set of 100 nymphs of same stage and age were maintained to compare its results with those of acetone-treated (control).

In the first set of 100 nymphs, each nymph was topically treated with 2 ul acetone. These nymphs were not significantly affected when compared to untreated nymphs. Out of 100 treated nymphs 4 died during the same instar whereas death of 2 nymphs occured at the ensuing ecdysis. Later, 3 nymphs died during the 5th instar and 2 died at the following nymphal-adult moulting. The total loss up to adult emergence was 2% more than that of the untreated nymphs (Table 1). The morphology and longevity of both 4th and

5th instar treated nymphs was unaffected. The number of adults having malformed wings was even less (1%) than that in untreated insects (Table 2). The females emerged from such treated nymphs laid almost as many eggs as those emerged from the untreated nymphs. There was no significant effect on the fecundity ($t=1.020$, $P>0.05$) and fertility ($t=1.535$, $p>0.05$; Table - 3).

In the next generation (F_1) adult emergence was insignificantly reduced by 17% which was 5% less than that in untreated nymphs (22% ; Table-4). The morphology of both nymphs and adults as well as nymphal longevity were similar to that of untreated insects. The effect on fecundity ($t=1.010$, $p>0.05$) and fertility ($t=1.466$, $p>0.05$) was also insignificant (Table - 5).

In the second set of 100 nymphs, each 4th instar nymph was topically treated with 5.0 µg Muristerone/nymph. Subsequently 3 nymphs died during the present instar whereas, 6 died at the following ecdysis. Death of 3 nymphs occurred during the 5th instar and that of 2 nymphs at nymphal-adult moulting. The number of adults emerged from such treated nymphs was 3% less than that of the control (89%), i.e. acetone treated nymphs (Table -1). There was no morphological abnormality or change in longevity of both

4th and 5th instar nymphs. However, the number of adults bearing malformed wings was even less in adults (2.16%) emerged from the treated 4th instar nymphs as compared to those emerged from the control (acetone-treated) nymphs which also had 4.49% adults with such deformity (Table-2). Each of the affected female on the average laid 0.5% eggs less than that of control but the difference was statistically insignificant ($t=0.530$, $p > 0.05$). Fertility of the eggs laid by the affected females was insignificantly reduced ($t=0.972$, $p > 0.05$; Table 3).

In F_1 generation (i.e. the insects developed from the eggs of the females which emerged following the treatment of 4th instar nymphs) from 100 Ist instar nymphs (four replicates of 25 nymphs each) total nymphal mortality up to adult emergenc was 11% as compared to 22% in case of control (Table-4). Further the morphological form and longevity of the nymphs in different instars remained unchanged. Similarly, there was no effect on adult morphology. The affected females of F1 generation on the average laid almost equal number of eggs as compared to control. Thus there was no significant effect on the fecundity of such females ($t=1.386$, $p > 0.05$). Egg fertility ($t=1.894$, $p > 0.05$) was also not affected significantly as compared to that of control (Table-5).

In the third set of 100 4th instar nymphs which were individually applied with 1.0 µg Muristerone, 8 nymphs died during the same instar and 6 at the next nymphal moulting. Further, 3 nymphs died during the 5th instar whereas death of 3 nymphs occured at nymphal-adult ecdysis. Adult emergence was dropped by 9% as compared to that of the control (Table-1). Both morphological changes and longevity of either 4th or 5th instar nymphs remained unchanged. However, 9.26% more emerged adults were malformed as compared to those emerged from control nymphs (Table-2). The emerged females (affected) on the average laid 5.72% less eggs than that of the control ($t=1.817$, $p>0.05$) which was insignificant. Similarly, the fertility remained unaffected as compared to control ($t=1.281$, $p>0.05$; Table-3).

In F1 generation total nymphal mortality up to adult emergence was only 2% more than that in the control (Table-4). There was no change in the nymphal longevity. The affected females laid 2.51% more eggs as compared to the control, which was statistically insignificant ($t=1.384$, $p>0.05$). Fertility of eggs laid by such females was also affected insignificantly ($t=2.381$, $p>0.05$) as compared to control (Table-5).

In the fourth set of 100 4th instar nymphs which were treated with 2.0 µg Muristerone/nymph, 12 nymphs died during the same instar and 10 nymphs could not survive at the ensuing nymphal moulting. Mortality during the 5th instar and at nymphal-adult ecdysis was respectively 1 and 5. Finally the fall in adult emergence was 17% more as compared to control (Table-1). There was no change in the longevity of the nymphs. In comparison to control 13.57% of the affected adults had malformed wings (Table-2). Fecundity of the females emerged from the treated nymphs was reduced by 13.25% which was statistically significant as compared to control ($t=2.699$, $p<0.05$). Whereas, reduction in the fertility was insignificant ($t=0.82$, $p>0.05$; Table-3).

In F1 generation total nymphal mortality was 15% which was insignificant when compared with that (22%) in the control (Table-4). There was neither morphological change nor effect on the nymphal longevity. Effect on fecundity ($t=0.356$, $p>0.05$) and fertility ($t=1.670$, $p>0.05$) was also statistically insignificant as compared to control (Table-5).

In the 5th set of 100 4th instar nymphs each topically treated with 4.0 µg Muristerone, the number of mortality during the same instar and at the following nymphal ecdysis was 16 and 17 respectively. In the next instar 4 nymphs

died and 3 nymphs could not survive at the nymphal-adult moulting. The total loss up to adult emergence was 29% more than that in control (Table-1). The longevity of both 4th and 5th instar nymphs was respectively dropped by 12-18 hours and 16-20 hours. Among the emerged adults 25.41% had malformed wings as compared to control (Table-2). In comparison to control, fecundity of the females emerged from the treated 4th instar nymphs dropped by 24.30% which was statistically significant ($t=3.355$, $p<0.05$). However, reduction in the fertility was insignificant ($t=0.892$, $p>0.05$) as compared to control (Table-3).

In the F1 generation total nymphal mortality up to adult emergence was 13% which was statistically insignificant when compared to that of control (22%; Table 4). The longevity of the nymphs was not affected. Apparently there was no abnormality in the adults. The effect on the fecundity ($t=0.341$, $p>0.05$) and the fertility ($t=1.751$, $p>0.05$) of the affected females was statistically insignificant (Table-5).

In the sixth set, each nymph was applied with 8.0 µg Muristerone. Out of 100 treated nymphs at the 4th instar (24 hrs.) 20 nymphs died during the same instar whereas death of 29 nymphs occurred at the moulting to the 5th instar. In

the later instar and then at the next moulting mortality was 5 and 4 respectively. The fall in adult emergence was 47% as compared to that in control (Table-1). The longevity of 4th and 5th instar nymphs respectively decreased by 20-28 hrs. and 16-24 hrs. The number of malformed adults emerged from the treated nymphs was 43.18% more than that of the control (Table-2). The affected females on the average laid 45.08% eggs less than those by control females which was statistically significant ($t=5.480$, $p < 0.05$). Whereas reduction in the fertility of their eggs ($t=2.141$, $p > 0.05$; Table-3) was insignificant as compared to control.

In the F1 generation loss up to adult emergence was 19% as compared to 22% in the control (Table-4). The longevity of nymphs of either instar remained unchanged. Fecundity of the affected females declined by 9.33% which was statistically insignificant ($t=2.337$, $p > 0.05$). Whereas fertility dropped by 1.02% which was also insignificant ($t=1.587$, $p > 0.05$) as compared to control (Table-5).

(b) Effect of injection of Muristerone to the 4th instar nymphs of Dysdercus cingulatus :

One day old 100 nymphs of 4th instar D. cingulatus were separately injected with each of doses of Muristerone as mentioned before. A set of 100 nymphs of the same age and stage was injected with 2µl acetone only to serve as control. Similarly, an equal number of untreated 4th instar were maintained for comparison with acetone treated nymphs.

In the first set each of 100 nymphs was injected with 2 µl acetone only. Then 3 nymphs died during the same (4th) instar whereas death of 4 nymphs occured during the 5th instar. Further, 3 nymphs suffered mortality at nymphal-adult moulting. The total mortality up to adult emergence was 10% which was insignificant as compared to that in untreated nymphs (11%; Table-6). The longevity of both 4th and 5th instar nymphs remained unaffected. Percentage of malformed adults emerged from the acetone treated nymphs was insignificant as compared to 7.86% in untreated insects (Table-7). The females which emerged following the injection of acetone in the 4th instar nymphs, on the average laid 2.90% less eggs than those emerged from the untreated nymphs. The reduction in the fecundity was statistically insignificant (0.95%; $t=1.485, p > 0.05$) as compared to that of untreated nymphs. Similarly fertility of the eggs remained unaffected ($t=1.678$; $p >$ [illegible]; Table 8).

In the F_1 generation number of nymphs which suffered mortality before finally moulting to adults was 15% as compared to 18% in case of control (untreated)Table 9). There was no change either morphological or in longevity. Similarly, fall in the fecundity ($y=1.604$, $p>0.05$) and fertility ($t=1.770$, $p>0.05$) were statistically insignificant in comparison with control (Table 10).

In the second set of 100 4th instar nymphs injected with 0.5 ug Muristerone/nymph, 3 nymphs suffered mortality during the same instar and 9 nymphs died at the following nymphal moulting. Death of 5 nymphs occured at the final moulting to adult. Thus the total nymphal mortality up to adult emergence was 6% more as compared to the control (i.e. acetone-treated; Table-6). The number of adults having malformed wings was 254% more than that of the control (Table-7). The average number of eggs laid by the affected females was equal to those laid by the control (acetone-treated) females, which was statistically insignificant ($t=0$, $p>0.05$). Effect on fertility was also insignificant ($t=1.504$, $p>0.05$; Table-8).

In the F1 generation total nymphal mortality was 5% more than that of the control (Table-9). There was no malformation either in the affected nymphs or adults. The

longevity of the nymphs in either instars was also unaffected. There was no significant effect on the fecundity ($t=1.854$, $p > 0.05$) and fertility ($t=1.899$, $p > 0.05$) of the affected females (Table-10).

In the third set each nymph of 4th instar was injected with 1.0 µg Muristerone and then 6 nymphs died during the same instar whereas 9 nymphs could not survive at the ensuing nymphal moulting. Death of 2 nymphs occurred during the 5th instar and 6 nymphs died at the final ecdysis. The total mortality in the affected nymphs was 13% more than that of the control (Table-6). Malformation of wings in emerged adults was 17.26% more as compared to adults which emerged from control (acetone-treated) nymphs (Table-7). Fecundity of the affected females reduced by 9.13% which was statistically insignificant ($t=2.176$, $p > 0.05$) as compared to that of the control. Similarly, fall in the fertility was insignificant ($t=0.982$, $p > 0.05$; Table-8).

In the F1 generation total nymphal mortality was 6% more than that of the control (Table-9). Malformation or change in longevity was not found in either the nymphs or the adults. The females of F1 generation on the average laid insignificantly more eggs than that of the control ($t=1.228$, $p > 0.05$). Similarly, there was no significant change in the fertility ($t=1.307$, $p > 0.05$) of the eggs laid by the affected females (Table-10).

In the Fourth set each nymph was individually injected with 2.0 µg Muristerone. Out of 100 treated nymphs, 6 nymphs died during the same instar whereas death of 18 nymphs occured at the following nymphal ecdysis. In the remaining, 3 nymphs suffered mortality during the 5th instar and 5 nymphs died at the following nymphal-adult moulting. Thus death of affected nymphs was 22% more than that of the control (Table-6). Malformation only occured in affected adults and it was 21.31% more than that of the control (Table-7). The females emerged from the treated nymphs on the average laid 14.23% less eggs than that of control which was statistically significant ($t=2.660$, $p < 0.05$). Whereas, drop in eggs fertility ($t=1.609$, $p > 0.05$) was insignificant (Table-8).

In the F1 generation number of emerged adults was 3% less than that of the control (Table-9). There was no effect on either longevity of the nymphs of both the instars or on formation of adults. Effect on the fecundity of the females of F1 generation was insignificant ($t=1.904$, $p > 0.05$) as compared to control. Similarly, fertility also ($t=1.497$, $p > 0.05$) remained unaffected (Table-10).

In the 5th set when each of 100 4th instar nymphs was injected with 4.0 µg Muristerone, 11 nymphs suffered mortality during the same instar whereas 25 nymphs died at the following nymphal moulting. Death of 4 nymphs occurred during the 5th instar and that of 8 nymphs at the nymphal-adult ecdysis. The total loss up to adult emergence was 38% more than that of the control (Table-6). The longevity of both instars dropped by 12-18 hrs.. The number of malformed adults was 28.53% more among those emerged from the treated nymphs as compared to those emerged from the control (Table-7). Reduction in the fecundity of the affected females was 24.75% which was statistically significant ($t=3.980$, $p < 0.05$) as compared to control. On the other hand, fertility was affected ($t=1.669$, $p > 0.05$) insignificantly (Table-8).

In the F1 generation the total nymphal mortality was 3% more than that of the control (Table-9). Nymphal longevity remained unchanged. There was no malformation in emerged adults. Similarly, there was no significant reduction either in the fecundity ($t=1.073$, $p > 0.05$) or fertility ($t=1.687$, $p > 0.05$) of the affected females as compared to that of the control (Table-10).

In the sixth set, 100 nymphs of 4th instar were individually injected with 8.0 µg Muristerone. Death of 17 nymphs occurred during the same instar whereas 33 nymphs died

at the following nymphal moulting. In the remaining nymphs, 5 suffered mortality during the 5th instar and 11 nymphs died at the nymphal-adult ecdysis. Total loss in adult emergence was 56% more than that of the control (Table-6). No malformation of any kind was seen in the nymphs of either instar. However, their longevity was respectively decreased by 20-28 hrs. and 16-24 hours. As compared to control, the number of adults bearing malformed wings was 44.84% more in those emerged from the treated nymphs (Table-6). However, 4% of the affected nymphs moulted to a supernumerary instar. The females emerged from the treated nymphs on the average laid 49.62% less eggs than that of the control, which was statistically significant ($t=5.967$, $p<0.05$). On the other hand, there was 5.40% reduction in the fertility ($t=1.810$, $p>0.05$) which was insignificant (Table-3).

In the F1 generation total loss in adult emergence was 9% more than that of the control (Table-9). The longevity of the nymphs was unchanged. Similarly, effect on the fecundity ($t=2.440$, $p>0.05$) and fertility ($t=2.030$, $p>0.05$) was statistically insignificant.

(c) Effect of topical application of Muristerone on the 5th instar nymphs of Dysdercus cingulatus:

As mentioned before, each of the selected sub-lethal dose of Muristerone was also topically applied on 100 5th instar nymphs (24-hrs. old) of Dysdercus cingulatus individually in four replicates, each consisting of 25 nymphs. Similarly, a set of 100 nymphs of the same instar and age were treated with 2 µl acetone to serve as control. An equal number of untreated nymphs was also maintained.

In the first set of 100 nymphs each nymph was topically treated with 2.0 µl acetone only. Then 4 nymphs died during the same instar and 2 died at the nymphal-adult ecdysis. Total nymphal mortality was 3% more than that of the untreated nymphs (Table-11). There was no change in the longevity of the nymphal instar. The percentage of malformed adults emerged from the acetone-treated nymphs was however, insignificantly (2.16%) more than that of untreated ones (Table-12). The females emerged from the treated nymphs did not exhibit significant change either in the fecundity ($t=1.629$, $p > 0.05$) or fertility ($t=1.493$, $p > 0.05$) as compared to those from untreated nymphs (Table-13).

In the F1 generation of the acetone-treated 5th instar nymphs the total nymphal mortality up to adult emergence was 7% more than that of the untreated nymphs (Table-14). The longevity of the nymphs was unaffected. Similarly, there was no occurrence of malformed adults as compared to control. The fecundity ($t=1.383$, $p > 0.05$) and fertility ($t=2.041$, $p > 0.05$) of the females of F1 generation were unaffected (Table-15).

In the second set each 5th instar nymph was applied with 0.5 µg Muristerone. Out of these 3 nymphs died during the same instar whereas death of 8 nymphs occurred at the ensuing nymphal-adult moulting. The loss in the number of adults emerged from the treated nymphs was 5% more than that of the control i.e., acetone-treated (Table-11). The number of the affected adults with malformed wings was 1.48% more than that of the control (Table-12). There was 6.50% fall in the fecundity of the affected females which was statistically insignificant ($t=1.951$, $p > 0.05$). Similarly fertility was also only insignificantly affected ($t=1.204$, $p > 0.05$; Table-13).

In the F1 generation total loss up to adult emergence was 2% less than that of the control (Table-14). There was no significant effect on the fecundity ($t=1.495$, $p > 0.05$) and fertility ($t=1.336$, $p > 0.05$) as compared to control (Table-15).

In the third set when each nymph was treated with 1.0 µg Muristerone, 6 nymphs died during the same instar whereas mortality of 12 nymphs occurred at the following nymphal-adult moulting. The total nymphal mortality in treated nymphs was 12% more than that of the control i.e. acetone-treated (Table-11). The number of malformed adults was 9.47% more in those emerged from the treated nymphs than that of the control (Table-12). The affected females on the average laid significantly less eggs (11.70%) than that of the control ($t=2.716$, $p < 0.05$). On the other hand, reduction in the egg fertility ($t=1.529$, $p > 0.05$) was insignificant (Table-13).

In the F1 generation nymphal mortality was 15% less than that of the control (Table-14). There was no change in the nymphal longevity and also no malformation was observed in the emerged adults. Similarly, the effect on the fecundity ($t=1.139$, $p > 0.05$) and fertility ($t=1.962$, $p > 0.05$) were insignificant (Table-15).

In the fourth set each nymph was applied with 2.0 µg Muristerone. It was followed by mortality of 12 and 11 nymphs in the same instar and at the next moulting respectively. Fall in the number of emerged adult emergence from treated nymphs was 17% more than that of the control

i.e. acetone-treated nymphs (Table-11). The number of malformed adults emerged from the treated nymphs was 19.59% more than that of the control which was significant (Table-12). The average number of eggs laid by the affected females was 20.70% less than that of the control. Thus the fall in the fecundity was significant ($t=4.013$, $p<0.05$) whereas that of fertility was insignificant ($t=2.176$, $p>0.05$) as compared to respective controls (Table-13).

In the F1 generation the number of nymphs which died before moulting to adults was 3% less in the affected nymphs than that of the control (Table-14). The longevity of the nymphs was unaffected. The females of F1 generation did not exhibit significant change in either fecundity ($t=1.612$, $p>0.05$) or fertility ($t=1.573$, $p>0.05$) as compared to control (Table-15).

In the fifth set, topically treated with 4.0 µg Muristerone, 17 nymphs suffered mortality during the 5th instar whereas 13 nymphs died at the following nymphal-adult moulting. Adult emergence dropped by 24% as compared to that of the control i.e. acetone-treated (Table-11). The longevity of the treated 5th instar nymphs dropped by 20-28 hours. The nymphs which moulted to the supernumerary instar

were 6% of the total treated nymphs. Following emergence, adults with malformed wings were 30.70% more than that of the control (Table-12). The affected females on the average laid 35.20% less eggs than that of the control which was statistically significant ($t=4.909$, $p<0.05$) whereas reduction in the fertility was insignificant ($t=2.176$, $p>0.05$) Table-13).

In the F1 generation loss of nymphs up to adult emergence was 3% more than that of the control (Table-14). Nymphal longevity was unaffected. Further, the fall in the fecundity ($t=1.661$, $p>0.05$) and fertility ($t=1.414$, $p>0.05$) were statistically insignificant as compared to the respective controls (Table-15).

In the sixth set, each of 100 nymphs of 5th instar was applied with 8.0 µg Muristerone. It was followed by the death of 21 nymphs during the same instar whereas, 20 nymphs suffered mortality at the ensuing nymphal-adult ecdysis. Total loss of life up to adult emergence was 35% more in the treated nymphs than that of the control i.e. acetone-treated (Table-11). The longevity of the treated nymphs shortened by 24-30 hours. Then 16% of the nymphs moulted to a supernumerary instar. The number of adults bearing malformed wings was 46.16% more among those emerged from the treated nymphs than those of the control (Table-12).

The fecundity of the affected females on the average decreased as compared to that of control ($t=6.799$, $p < 0.05$). The reduction in the fertility was 12.83% which was also significant ($t=2.780$, $p < 0.05$; Table-13).

In the F1 generation total nymphal mortality was 8% more in the affected nymphs than in the control (Table-14). Nymphal longevity was unchanged. The affected females on the average laid 18.02% less eggs than that of the control which was statistically significant ($t=3.689$, $p < 0.05$). But 0.34% reduction in the fertility ($t=1.459$, $p > 0.05$) was insignificant (Table-15).

(d) Effect of injection of Muristerone to the 5th instar nymphs of *Dysdercus cingulatus*:

Like the injection of sub-lethal doses of Muristerone to the 4th instar nymphs of *D. cingulatus*, 5th instar nymphs were also individually injected with each dose to 100 nymphs in four replicates each consisting of 25 nymphs. An equal number of nymphs of same stage and age which were injected with 2 µl acetone only served as control and another set of 100 untreated nymphs were also maintained.

In the first set only acetone (2 µl/nymph) was injected and then 3 nymphs died during the same instar whereas 2 nymphs died at the nymphal-adult ecdysis. The mortality among the treated nymphs was almost equal to that of untreated nymphs (Table-16). The number of malformed adults was 1% less with respect to acetone applicates than that of the untreated nymphs (Table-17). There was no significant drop either in fecundity ($t=1.457, p > 0.05$) or fertility ($t=1.808, p > 0.05$) of the affected females in comparison to that of the untreated insects (Table-18).

In the F1 generation total nymphal mortality was 2% less in the progeny of acetone-treated nymphs than in that of untreated (Table-19). The effect on the fecundity ($t=0.620, p > 0.05$) and fertility ($t=1.248, p > 0.05$) was insignificant (Table-20).

In the second set, each 5th instar nymph was injected with 0.5 µg Muristerone. Thereafter 3 nymphs died during this instar whereas 6 nymphs could not survive at the following nymphal-adult moulting. There was 4% more drop in adult emergence as compared to that of the control i.e., acetone-treated (Table-16). Neither malformation nor change in the longevity of the treated nymphs was seen. The number of adults having malformed wings was 6.78% more

among those emerged from the treated nymphs than the control (Table-17). Fecundity ($t=2.317$, $p>0.05$) and fertility ($t=1.540$, $p>0.05$) of the affected females dropped insignificantly as compared to the respective controls (Table-18).

In the F1 generation of the 5th instar nymphs injected with Muristerone (0.5 ug/nymph) nymphal mortality was rather 4% less than that of the control (Table-19). Fecundity ($t=1.047$, $p>0.05$) and fertility ($t=1.337$, $p>0.05$) was affected only insignificantly (Table-20).

In the third set of 5th instar nymphs each nymph injected with 1.0 ug Muristerone, 5 nymphs died during the same instar and 9 nymphs could not survive at the ensuing nymphal-adult ecdysis. The loss of life up to adult emergence was 9% more than that of the control i.e., acetone-treated (Table-16). Among the adults emerged from the treated nymphs the nymphs with malformed wings were 9.7% more than that of the control (Table-17). The affected females on the average laid 16.38% less eggs than the control which was statistically significant ($t=3.155$ $p<0.05$) on the other hand, fall in the fertility was insignificant ($t=1.747$, $p>0.05$); Table-18).

In the F1 generation there was 4% higher mortality in the progeny of the affected nymphs than that of the control

(Table-19). There was no significant change in the fecundity ($t=0.998$, $p>0.05$) and fertility ($t=1.734$, $p>0.05$)of the affected females (Table-20).

In the fourth set, 2.0 µg Muristerone was injected in each of 100 nymphs. Among them 10 nymphs died during the same instar whereas mortality of 12 nymphs occurred at nymphal-adult moulting. The reduction in the adult number was 17% more than that of the control i.e., acetone-treated (Table-16). The number of malformed adults was 25.28% higher among those emerged from the treated nymphs than that of the control (Table-17). The affected females on the average laid 28.20% less eggs than that of the control. Thus drop in the fecundity was statistically significant as compared to control females ($t=4.522$, $p<0.05$). But the fertility ($t=2.425$, $p>0.05$) was only insignificantly reduced (Table-18).

In the F1 generation total nymphal mortality was 1% more than that of the control (Table-19). The fecundity ($t=0.990$, $p>0.05$) and fertility ($t=1.259$, $p>0.05$) of the emerged females remained unaffected.

In the fifth set each nymph was injected 4.0 µg Muristerone. Thereafter, 18 nymphs suffered mortality during the same instar whereas 14, nymphs died during the

nymphal-adult moulting. Among the treated nymphs there was 27% higher mortality than that of the control i.e. acetone-treated (Table-16). Among the treated nymphs 3% moulted to a supernumerary instar. There were 34.03% adults bearing malformed wings higher than that of the control (Table-17). The females emerged from the treated nymphs on the average laid 47.87% less eggs than that of the control, which was statistically significant ($t=4.948$, $p < 0.05$). On the other hand, fall in the fertility was 5.88% which was insignificant ($t=1.778$, $p > 0.05$) as compared to control (Table-18).

In the F1 generation of such treated nymphs adult emergence was 2% less than that of the control (Table-19). Further, there was statistically significant reduction in the fecundity ($t=2.202$, $p > 0.05$) and fertility ($t=1.941$, $p > 0.05$) as compared to the respective control (Table-20).

In the sixth set when each nymph was injected with 8.0 µg Muristerone death of 24 nymphs occurred during the same instar whereas, 24 nymphs suffered mortality at the ensuing moulting. The drop in adult emergence was 43% higher among those emerged from the treated nymphs than that of the control i.e. acetone-treated (Table-16). But 11% of the treated nymphs moulted to a supernumerary instar.

The longevity of the treated nymphs shortened by 24-32 hours. The adults with malformed wings were 53.48% more than those of the control (Table-17). The fecundity of the affected females on the average dropped by 64.15% which was statistically significant ($t=6.967$, $p < 0.05$) as compared to the control. Similarly, reduction in the fertility was 11.75% which was also statistically significant ($t=2.963$, $p < 0.05$) as compared to the control (Table-18).

In the F1 generation there was considerable loss before adult emergence which was 14% more than that of the control (Table-19). The affected females on the average laid 17.91% less eggs than that of the control which was significant ($t=3.419$ $p < 0.05$) whereas reduction in the fertility (1.57%) was statistically insignificant ($t=1.697$, $p > 0.05$) as compared to control (Table-20).

Table - 1: Showing nymphal mortality and adult emergence following the topical application of different doses of Muristerone on the 4th instar nymphs (24 hrs old) of Dysdercus cingulatus.

Doses (µg/nymph)	Nymphal mortality				Adult emergence
	4th instar	At moulting (4th-5th instar	5th instar	At nymphal-adult moulting	
0.5	3	6	3	2	86
1.0	8	6	3	3	80
2.0	12	10	1	5	72
4.0	16	17	4	3	60
8.0	20	29	5	4	42
Control (Acetone-treated)	4	2	3	2	89
Untreated	3	2	1	3	91

Total 100 treated nymphs/dose, 4 replicates of 25 each.

Table 2: Showing the percentage of malformed adults emerged from 4th instar nymphs (24 hrs. old) of Dysdercus cingulatus topically treated with different doses of Muristerone.

Doses (µg/nymph)	Total no. of emerged adults	No. of adults with malformed wings	Percentage of adults with malformed wings
0.5	86	2	2.33
1.1	80	11	13.75
2.0	72	13	18.06
4.0	60	18	30.00
8.0	42	20	47.62
Control (Acetone-treated)	89	4	4.49
Untreated	91	5	5.49

Total 100 treated nymphs/dose, 4 replicates of 25 each.

Table 3: Showing fecundity and fertility of Dysdercus cingulatus females emerged following the topical application of different doses of Muristerone on the 4th instar nymphs (24 hrs. old).

Doses (µg/nymph)	No. of eggs laid (mean ± S.E.)	No. of eggs hatched (mean ± S.E.)	Percent hatching
0.5	165.17 ± 4.03	162.17 ± 3.94	98.18
1.0	156.50 ± 3.83	151.17 ± 3.95	96.08
2.0	144.00 ± 4.18	141.67 ± 4.29	98.38
4.0	125.67 ± 5.55	121.83 ± 6.27	96.94
8.0	91.17 ± 2.88	80.00 ± 3.08	87.75
Control(Acetone-treated)	166.00 ± 3.22	159.50 ± 3.55	96.08
Untreated	168.50 ± 2.66	162.50 ± 2.93	96.44

Table- 4: Showing nymphal mortality and adult emergence in F_1 generation following the topical application of different doses of Muristerone on the 4th instar nymphs (24 hrs old) of F generation of Dysdercus cingulatus.

Doses (µg/nymph)	Nymphal mortality					Adult emergence
	Ist instar	2nd instar	3rd instar	4th instar	5th instar	
0.5	5	3	3	-	-	89
1.0	7	8	1	2	6	76
2.0	6	6	3	-	-	85
4.0	3	1	-	4	5	87
8.0	7	2	-	2	7	82
Control)Acetone-treated)	10	5	5	2	-	78
Untreated	2	4	7	1	3	83

Total 100 Ist instar nymphs/dose, 4 replicates of 25 each.

Table 5 : Showing fecundity and fertility of Dysdercus cingulatus females (F_1 generation) following topical application of different doses of Muristerone on the 4th instar nymphs (24 hr old) of F generation.

Doses (µg/nymph)	No. of eggs laid (mean±S.E.)	No. of eggs hatched (mean±S.E.)	Percent hatching
0.5	179.50 ± 2.40	170.83 ± 3.52	95.17
1.0	179.50 ± 2.42	170.50 ± 1.48	94.99
2.0	174.67 ± 3.05	167.00 ± 3.69	95.61
4.0	175.33 ± 3.60	166.17 ± 3.72	94.78
8.0	158.67 ± 3.99	151.00 ± 3.47	95.17
Control(Acetone-treated)	175.00 ± 3.34	168.33 ± 4.26	96.19
Untreated	177.50 ± 2.67	171.50 ± 2.69	96.62

Table 6: Showing nymphal mortality and adult emergence following the injection of different doses of Muristerone into the 4th instar nymphs (24 hrs.old) of *Dysdercus cingulatus*.

Doses (µg/nymph)	Nymphal Mortality				Adult emergence
	4th instar	At moulting (4th-5th instar)	5th instar	At nymphal-adult moulting	
0.5	3	8	-	5	84
1.0	6	9	2	6	77
2.0	6	18	3	5	68
4.0	11	25	4	8	52
8.0	17	33	5	11	34
Control(Acetone-treated)	3	-	4	3	90
Untreated	4	3	4	-	89

Total 100 treated nymphs/dose, 4 replicates of 25 each.

Table 7 : Showing the percentage of malformed adults emerged from 4th instar nymphs (24 hrs old) of Dysdercus cingulatus , injected with different doses of Muristerone.

Doses (µg/nymph)	Total no. of emerged adults	No. of adults with malformed adults	Percentage of adults with malformed wings
0.5	84	4	4.76
1.0	77	15	19.48
2.0	68	16	23.53
4.0	52	16	30.75
8.0	34	16	47.06
Control(Acetone-treated)	90	2	2.22
Untreated	89	7	7.86

Total 100 treated nymphs/dose, 4 replicates of 25 each.

Table 8: Showing fecundity and fertility of Dysdercus cingulatus females emerged from 4th instar nymphs (24 hrs old) injected with different doses of Muristerone.

Doses (µg/nymph)	No. of eggs laid (mean ± S.E.)	No. of eggs hatched (mean value ±S.E.)	Percent hatching
0.5	173.33 ± 3.11	167.50 ± 2.79	96.63
1.0	162.17 ± 2.80	159.67 ± 3.56	98.46
2.0	152.17 ± 4.34	146.33 ± 4.50	96.17
4.0	132.17 ± 3.38	124.83 ± 3.07	94.45
8.0	87.33 ± 2.93	79.17 ± 3.16	90.66
Control(Acetone-treated)	173.33 ± 2.98	166.50 ± 2.96	96.06
Untreated	178.50 ± 2.76	173.17 ± 2.79	97.01

Table 9 : Showing nymphal mortality and adult emergence in F_1 generation following the injection of different doses of Muristerone into the 4th instar nymphs (24 hrs. old) of F generation of Dysdercus cingulatus.

Doses (µg/nymph)	Nymphal mortality					Adult emergence
	Ist instar	2nd instar	3rd instar	4th instar	5th instar	
0.5	6	3	6	3	2	80
1.0	7	6	-	3	5	79
2.0	3	6	3	6	-	82
4.0	5	4	5	3	1	82
8.0	9	6	4	3	2	76
Control(Acetone-treated)	4	-	2	6	3	85
Untreated	4	6	5	3	-	82

Total 100 instar nymphs/dose, 4 replicates of 25 each.

Table 10: Showing fecundity and fertility of Dysdercus cingulatus females (F_1 generation) injected with different doses of Muristerone into 4th instar nymphs (24 hrs old) of F generation.

Doses (µg/nymph)	No. of eggs laid (mean ± S.E.)	No. of eggs hatched (mean ± S.E.)	Percent hatching
0.5	173.83 ± 1.45	170.00 ± 1.15	97.78
1.0	172.33 ± 3.26	167.67 ± 3.43	97.30
2.0	176.60 ± 3.15	171.50 ± 2.42	97.11
4.0	167.17 ± 2.39	161.17 ± 2.70	96.41
8.0	156.50 ± 3.34	147.17 ± 2.20	94.04
Control(Acetone-treated	169.17 ± 1.87	163.67 ± 2.43	96.75
Untreated	175.17 ± 3.84	171.17 ± 3.49	97.72

Table 11: Showing nymphal mortality and adult emergence following the topical application of different doses of Muristerone on the 5th instar nymphs (24 hrs old) of Dysdercus cingulatus.

Doses (µg/nymph)	Nymphal mortality		Adult emergence
	5th instar	At nymphal-adult moulting	
0.5	3	8	89
1.0	6	12	82
2.0	12	11	77
4.0	17	13	70
8.0	21	20	59
Control(Acetone-treated)	4	2	94
Untreated	3	-	97

Total 100 treated nymphs/dose , 4 replicates of 25 each.

Table 12 : Showing percentage of malformed adults emerged from 5th instar nymphs (24 hrs. old) of Dysdercus cingulatus, topically treated with different doses of Muristerone.

Doses (µg/nymph)	Total no. of emerged adults	No. of adults with malformed wings	Percentage of adults with malformed wings
0.5	89	7	7.86
1.0	82	13	15.85
2.0	77	20	25.97
4.0	70	26	37.14
8.0	59	31	52.54
Control(Acetone-treated)	94	6	6.38
Untreated	97	4	4.12

Total 100 treated nymphs/dose, 4 replicates of 25 each.

Table 13: Showing fecundity and fertility of *Dysdercus cingulatus* females emerged from 5th instar nymphs (24 hrs. old) topically treated with different doses of Muristerone.

Doses (µg/nymph)	No. of eggs laid (mean ± S.E.)	No. of eggs hatched (mean ± S.E.)	Percent hatching
0.5	155.83 ± 3.99	150.83 ± 4.45	96.79
1.0	147.17 ± 3.49	141.83 ± 2.10	96.37
2.0	132.17 ± 2.27	124.17 ± 1.92	93.95
4.0	108.00 ± 2.98	98.00 ± 2.19	90.74
8.0	71.83 ± 2.05	59.83 ± 1.76	84.47
Control(Acetone-treated)	166.67 ± 2.98	162.17 ± 2.48	97.30
Untreated	173.33 ± 3.17	170.17 ± 2.38	98.18

Table 14 : Showing nymphal mortality and adult emergence in F_1 generation following the topical application of different doses of Muristerone on the 5th instar nymphs (24 hrs old) of F generation of Dysdercus cingulatus.

Doses (µg/nymph)	Nymphal Mortality					Adult emergence
	Ist instar	2nd instar	3rd instar	4th instar	5th instar	
0.5	3	3	6	4	3	81
1.0	4	-	-	2	-	94
2.0	6	5	5	-	2	82
4.0	8	8	6	-	2	76
8.0	12	8	3	3	3	71
Control(Acetone-treated)	7	4	6	2	2	79
Untreated	6	5	-	3	-	86

Total 100 Ist instar nymphs/dose, 4 replicates of 25 each.

Table 15 : Showing fecundity and fertility of Dysdercus cingulatus females (F_1 generation) topically treated with different doses of Muristerone on the 5th instar nymphs (24 hrs. old) of F generation.

Doses (µg/nymph)	No. of eggs laid (mean ± S.E.)	No. of eggs hatched (mean ± S.E.)	Percent hatching
0.5	167.67 ± 3.29	163.33 ± 2.66	97.41
1.0	177.83 ± 1.60	169.50 ± 1.34	95.32
2.0	175.83 ± 2.51	171.00 ± 3.26	97.25
4.0	174.33 ± 3.65	168.67 ± 3.28	96.75
8.0	147.83 ± 4.17	140.50 ± 4.27	95.04
Control(Acetone-treated)	180.33 ± 1.69	172.00 ± 3.21	95.38
Untreated	177.17 ± 2.36	172.00 ± 2.42	97.08

Table 16 : Showing nymphal mortality and adult emergence following the injection of different doses of Muristerone into the 5th instar nymphs (24 hrs. old) of Dysdercus cingulatus.

Doses (µg/nymph)	Nymphal mortality		Adult emergence
	5th instar	At nymphal-adult moulting	
0.5	3	6	91
1.0	5	9	86
2.0	10	12	78
4.0	18	14	68
8.0	24	24	52
Control(Acetone-treated)	3	2	95
Untreated	-	4	96

Total 100 treated nymphs/dose, 4 replicates of 25 each.

Table 17 : Showing percentage of malformed adults emerged from 5th instar nymphs (24 hrs. old) of Dysdercus cingulatus injected with different doses of Muristerone.

Doses (µg/nymph)	Total no. of emerged adults	No. of adults with malformed wings	Percentage of adults with malformed wings
0.5	91	10	10.99
1.0	86	12	13.95
2.0	78	23	29.49
4.0	68	26	38.24
8.0	52	30	57.69
Control(Acetone-treated)	95	4	4.21
Untreated	96	5	5.21

Total 100 treated nymphs/dose, 4 replicates of 25 each.

Table 18: Showing fecundity and fertility of Dysdercus cingulatus females emerged from 5th instar nymphs (24 hrs. old) injected with different doses of Muristerone.

Doses (µg/nymph)	No. of eggs laid (mean ± S.E.)	No. of eggs hatched (mean ± S.E.)	Percent hatching
0.5	157.67 ± 2.83	153.17 ± 1.82	97.15
1.0	143.83 ± 3.23	136.50 ± 2.66	94.90
2.0	123.50 ± 2.10	113.83 ± 1.93	92.17
4.0	89 .67 ± 4.53	79.17 ± 3.73	88.29
8.0	61.67 ± 1.86	50.83 ± 1.16	82.42
Control(Acetone-treated)	172.00 ± 3.71	162.00 ± 3.78	94.17
Untreated	165.50 ± 3.78	160.83 ± 3.49	97.18

Table 19 : Showing nymphal mortality and adult emergence in F_1 generation following the injection of different doses of Muristerone into the 5th instar nymphs (24 hrs. old) of F generation of Dysdercus cingulatus.

Doses (µg/nymph)	Nymphal Mortality					Adult emergence
	Ist instar	2nd instar	3rd instar	4th instar	5th instar	
0.5	3	7	-	2	2	86
1.0	4	9	6	3	3	78
2.0	5	7	5	2	-	81
4.0	6	4	1	5	4	80
8.0	12	7	3	5	5	68
Control(Acetone-treated)	5	2	4	4	3	82
Untreated	6	-	5	7	2	80

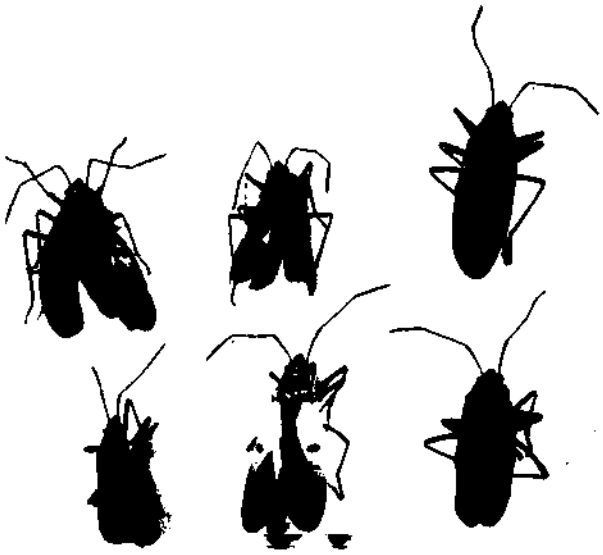
Total 100 Ist instar nymphs/dose, 4 replicates of 25 each.

Table 20: Showing fecundity and fertility of Dysdercus cingulatus females (F_1 generation) injected with different doses of Muristerone into the 5th instar nymphs (24 hrs. old) of F generation.

Doses (µg/nymph)	No. of eggs laid (mean ± S.E.)	No. of eggs hatched (mean ± S.E.)	Percent hatching
0.5	175.67 ± 3.64	170.00 ± 4.13	96.77
1.0	169.50 ± 2.39	164.00 ± 2.16	96.75
2.0	175.33 ± 3.55	170.33 ± 3.52	97.15
4.0	159.67 ± 2.14	153.17 ± 2.09	95.93
8.0	141.33 ± 2.28	135.00 ± 3.10	95.52
Control(Acetone-	172.17 ± 4.18	167.17 ± 3.67	97.09
Untreated	173.33 ± 3.20	169.00 ± 3.70	97.50

Fig. 1: Adult malformation in *Dysdercus cingulatus* following the treatment with Muristerone.

Fig. 2 & 3 : Failure of *Dysdercus cingulatus* nymphs in escaping from the exuviae during the ecdysis.

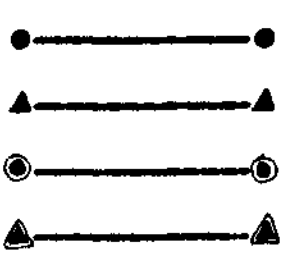


①

②

③

Fig. 1 : Showing total nymphal mortality up to adult emergence following the topical application and injection of different doses of Muristerone to the 4th and 5th instar nymphs of Dysdercus cingulatus respectively.



● = 4th instar.....Topical application

▲ = 4th instar.....Injection

◉ = 5th instar.....Topical application

▲ = 5th instar.....Injection

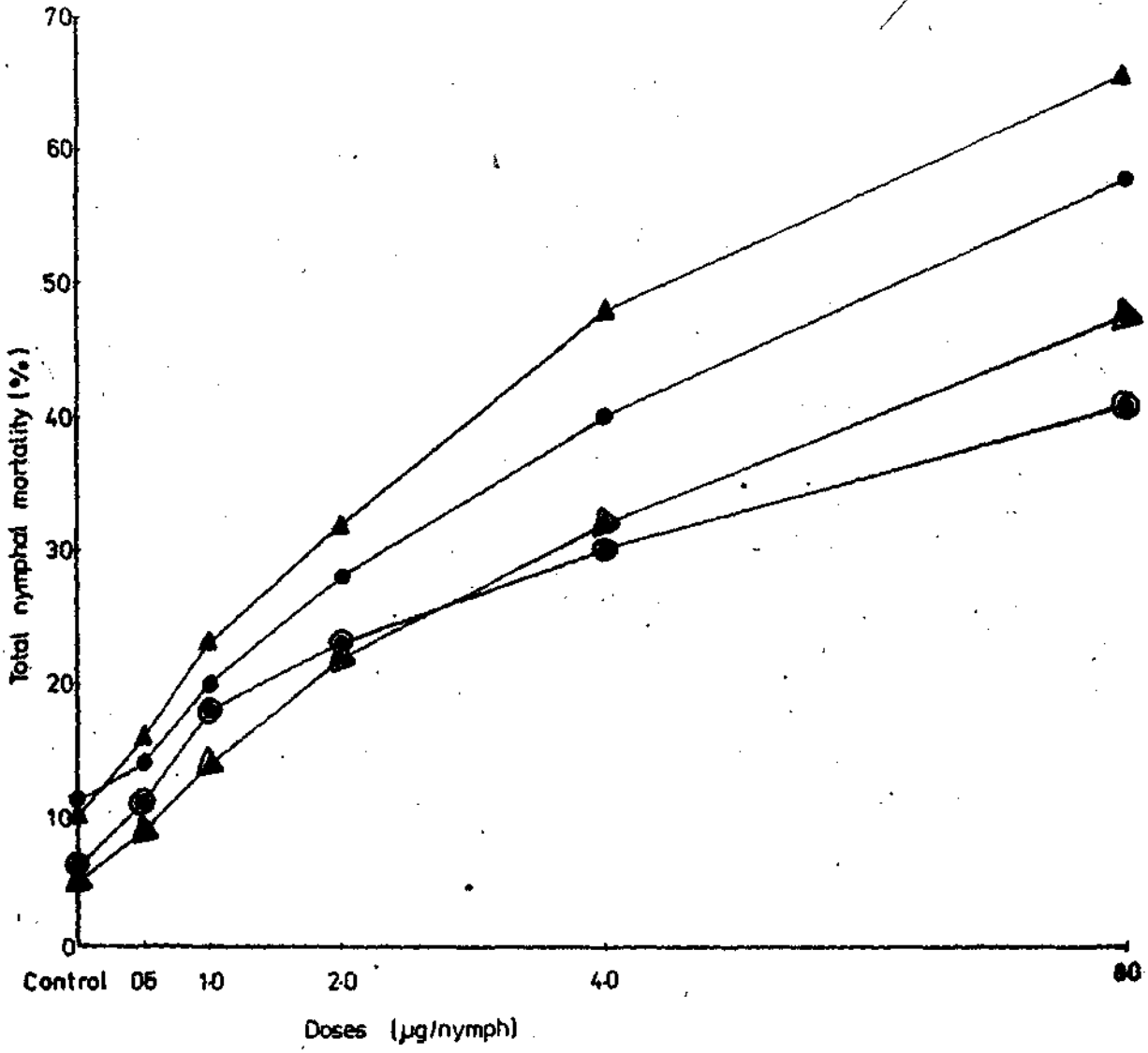
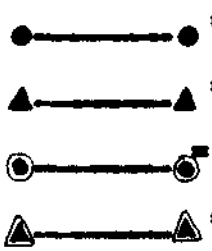

70
60
50
40
30
20
10
0
Total nymphal mortality (%)
Control
0·5
1·0
2·0
4·0
8·0
Doses (µg/nymph)

Fig. 2: Shewing percentage of malformed adults emerged following the topical application and injection of different doses of Muristerone to the 4th and 5th instar nymphs of Dysdercus cingulatus respectively.



● = 4th instar ..... Topical application

▲ = 4th instar ..... Injection

◉ = 5th instar ..... Topical application

△ = 5th instar ..... Injection

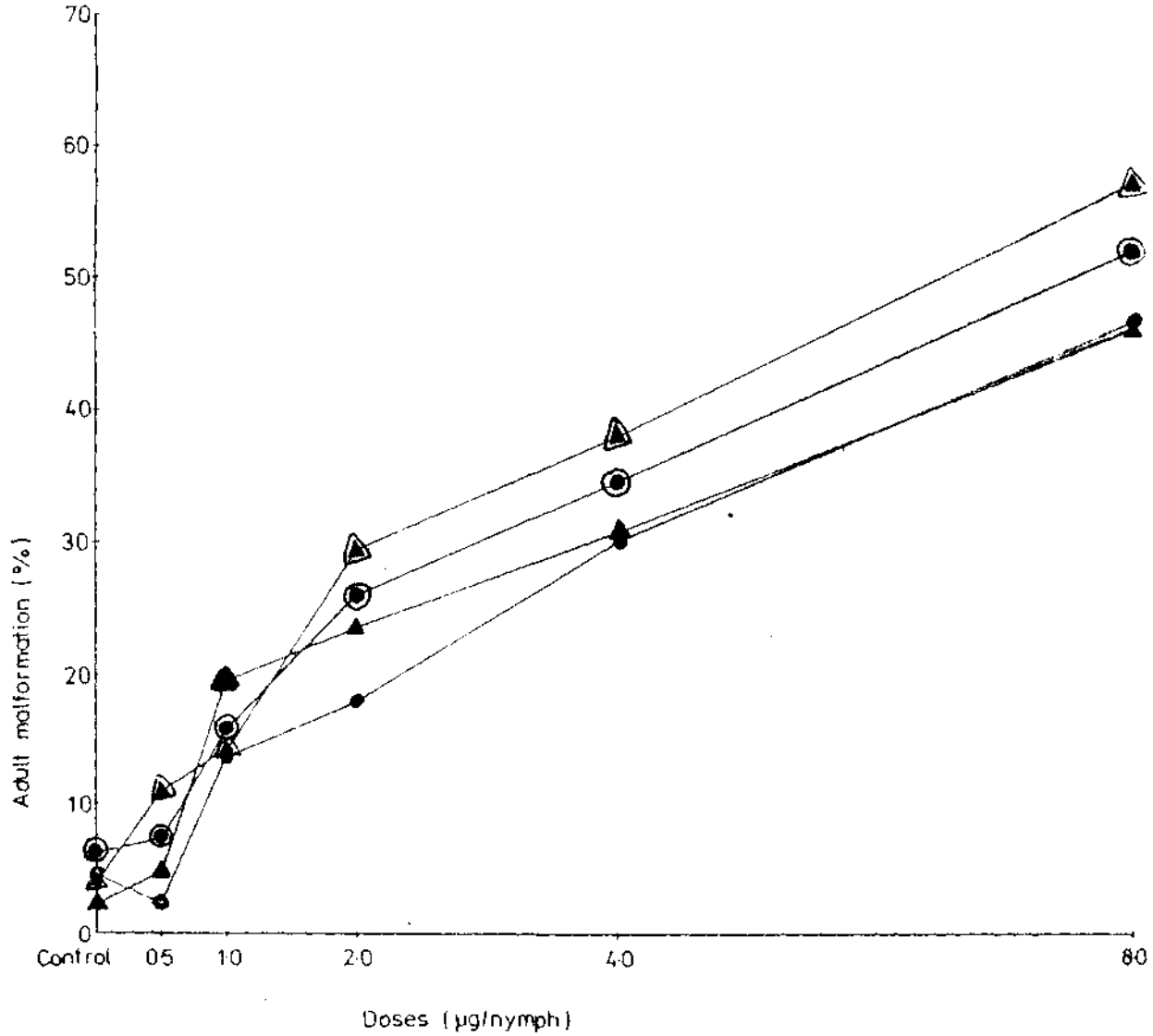
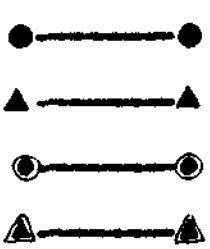

70
60
50
40
30
20
10
0
Adult malformation (%)
Control
05
10
2·0
4·0
8·0
Doses (µg/nymph)

Fig. 3: Shewing reduction(percentage) in the fecundity of female Dysdercus cingulatus emerged following the topical application and injection of different doses of Muristerone to the 4th and 5th instar nymphs respectively.



● ——— ● = 4th instar..... Topical application

▲ ——— ▲ = 4th instar..... Injection

◉ ——— ◉ = 5th instar..... Topical application

▲ ——— ▲ =5th instar.....Injection

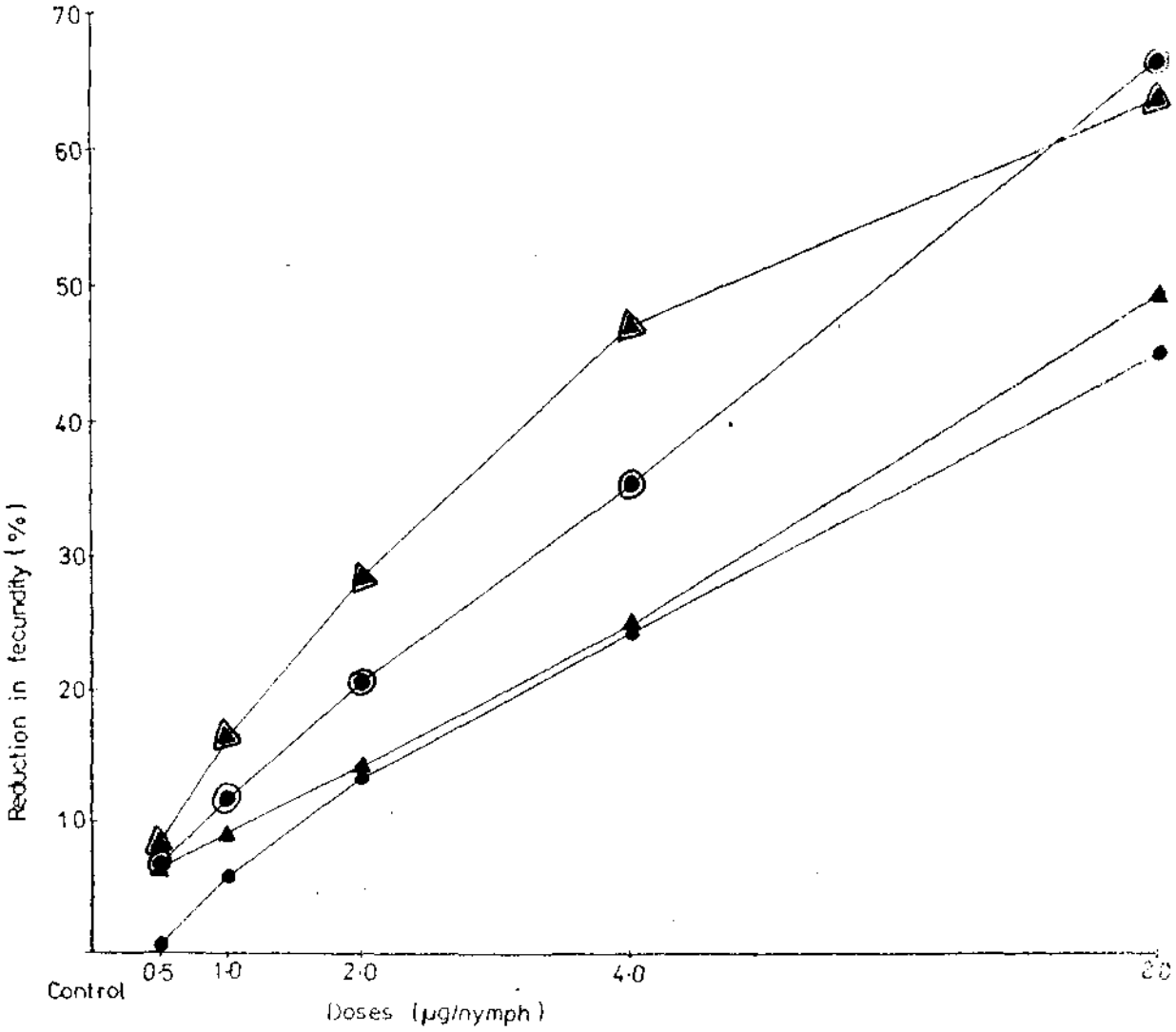

70
60
50
40
30
20
10
Reduction in fecundity (%)
Control
0·5
1·0
2·0
4·0
Doses (µg/nymph)

Fig. 4: Showing percentage of unhatched eggs(reduction in egg-fertility) of female Dysdercus cingulatus emerged following the topical application and injection of different doses of Muristerone to the 4th and 5th instar nymphs respectively.

●——● = 4th instar.....Topical application

▲——▲ = 4th instar.....Injection

◉——◉ = 5th instar.....Topical application

◭——◭ = 5th instar.....Injection

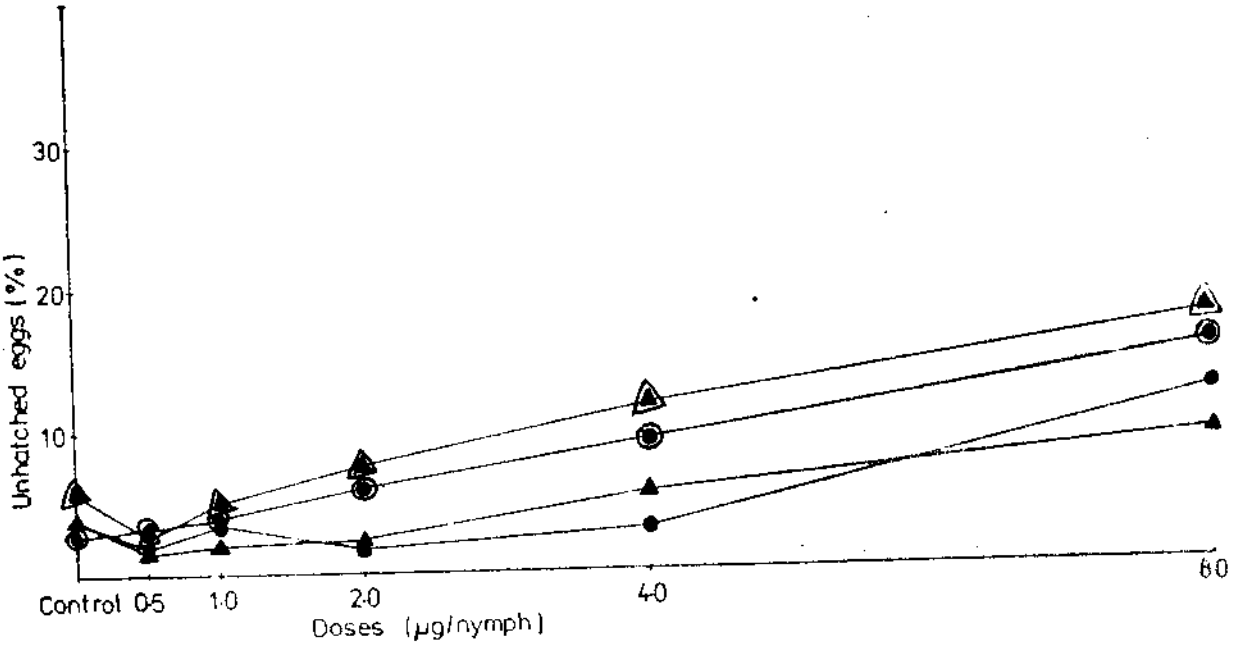

Unhatched eggs (%)
30
20
10
Control
0·5
1·0
2·0
4·0
6·0
Doses (µg/nymph)

Fig. 5: Showing total nymphal mortality up to adult emergence in F1 generation following topical application and injection of different doses of Muristerone in the 4th and 5th instar nymphs of Dysdercus cingulatus respectively in F generation.

● ——— ● = 4th instar.....Topical application

▲ ——— ▲ = 4th instar.....Injection

◐ ——— ◐ = 5th Instar..... Topical application

◭ ——— ◭ = 5th instar.....Injection

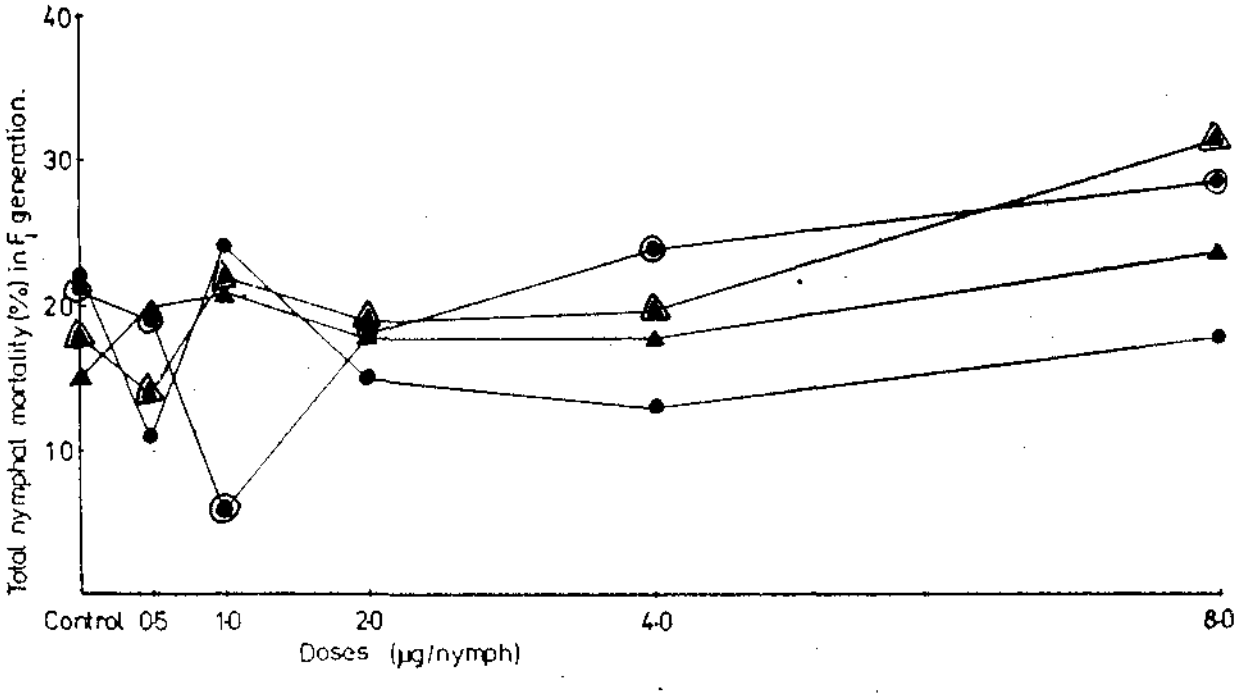

40
30
20
10
Total nymphal mortality (%) in F1 generation.
Control
05
1·0
2·0
4·0
8·0
Doses (µg/nymph)

Fig. 6: Showing total nymphal mortality up to adult emergence following the topical application and injection of different doses of Muristerone to 4th and 5th instar nymphs of Dysdercus cingulatus respectively.

A = 4th instar nymphs .....Topical application

B = 4th instar nymphs .....Injection

C = 5th instar nymphs .....Topical application

D = 5th instar nymphs .....Injection

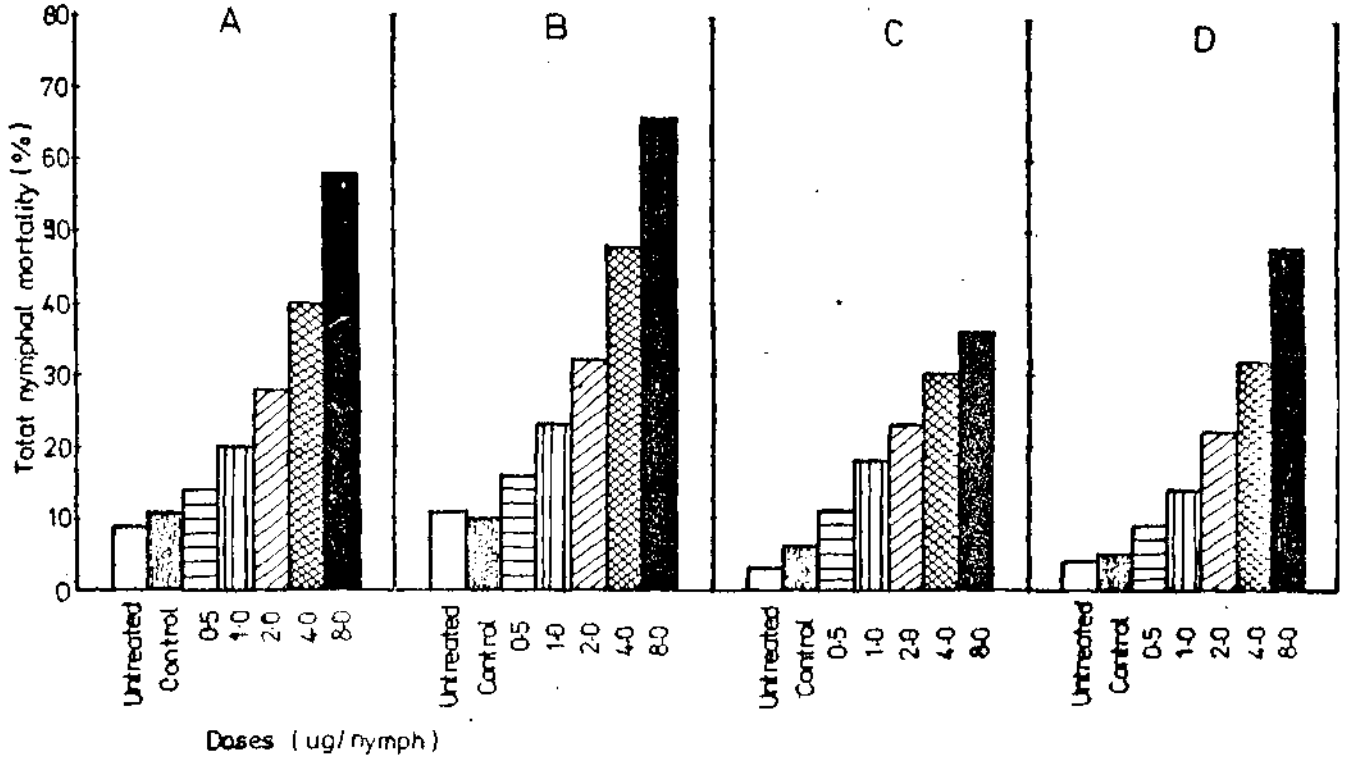

A
B
C
D
Total nymphal mortality (%)
0
10
20
30
40
50
60
70
80
Untreated
Control
0·5
1·0
2·0
4·0
8·0
Doses (ug/ nymph)

Fig. 7: Showing percentage of malformed adults emerged following the topical application and injection of different doses of Muristerone to the 4th and 5th instar nymphs of *Dysdercus cingulatus* respectively.

A = 4th instar nymphs.....Topical application

B = 4th instar nymphs.....Injection

C = 5th instar nymphs.....Topical application

D = 5th instar nymphs.....Injection

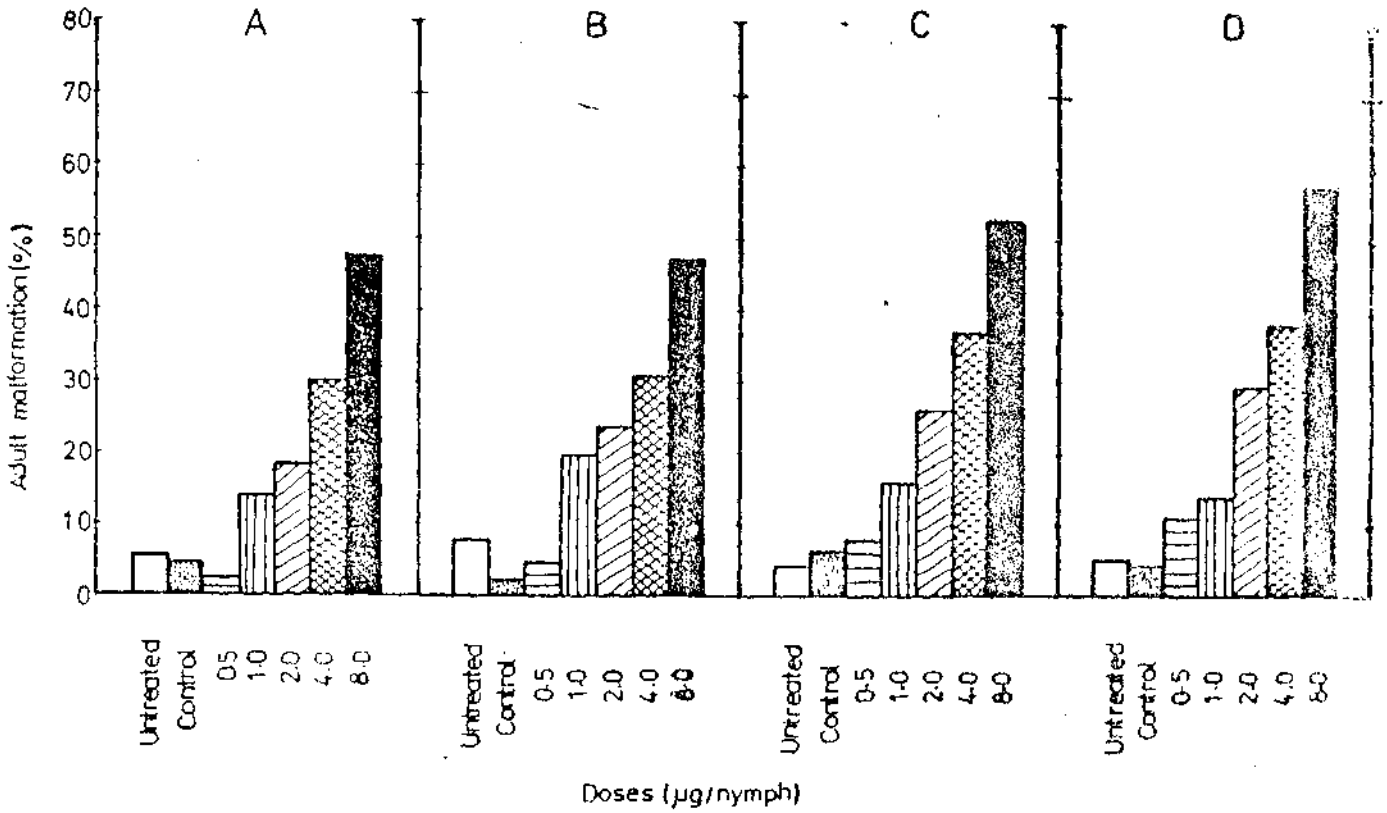

A
B
C
D
Adult malformation (%)
0
10
20
30
40
50
60
70
80
Untreated Control
0·5
1·0
2·0
4·0
8·0
Doses (µg/nymph)

Fig. 8: Showing reduction in the fecundity of female Dysdercus cingulatus emerged following the topical application and injection of different doses of Muristerone to the 4th and 5th instar nymphs respectively.

A = 4th instar nymphs .....Topical application

B = 4th instar nymphs.....Injection

C = 5th inster nymphs.....Topical applicatin

D = 5th instar nymphs .....Injection

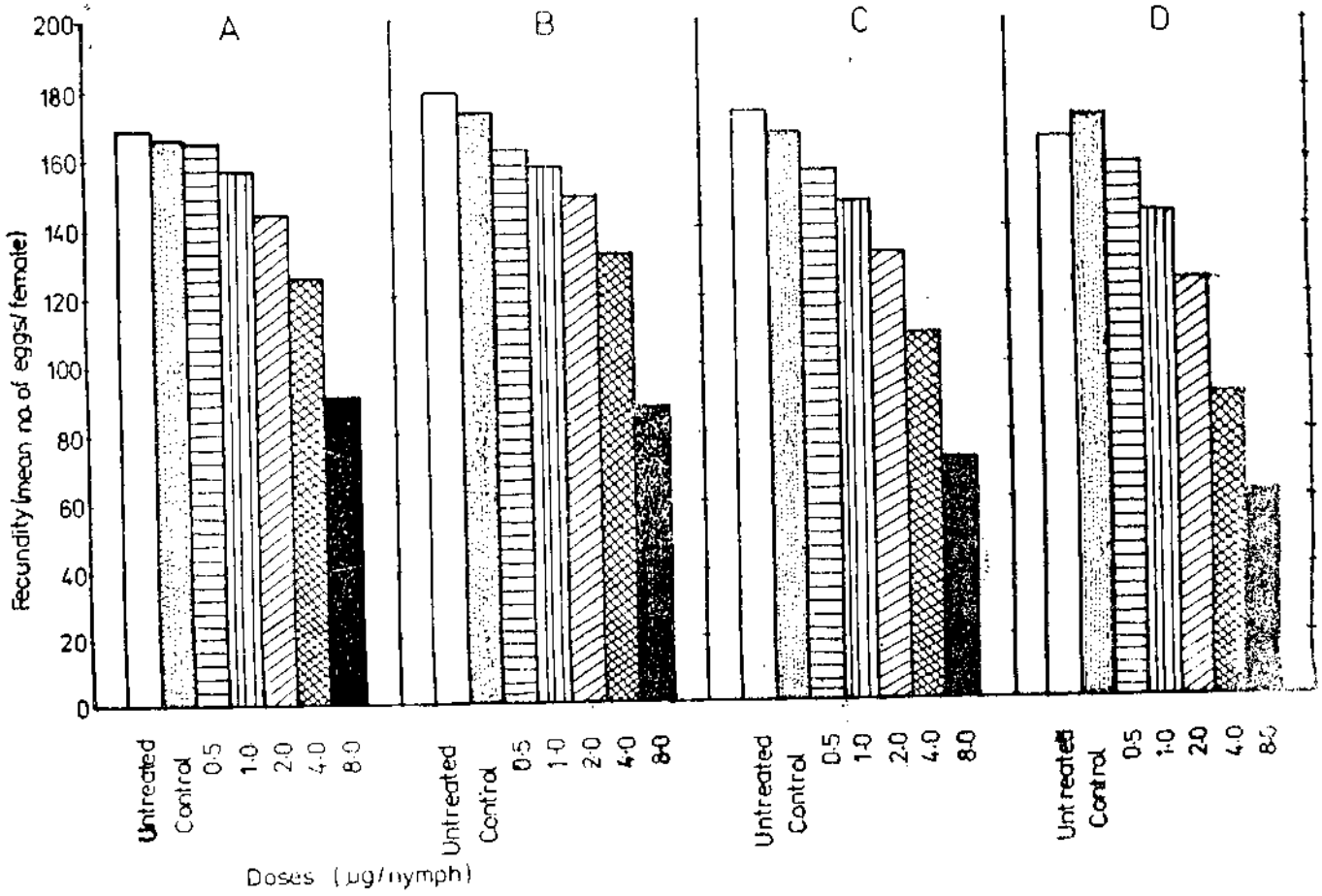

A
B
C
D
Fecundity (mean no of eggs/female)
200
180
160
140
120
100
80
60
40
20
0
Untreated Control
0·5
1·0
2·0
4·0
8·0
Doses (µg/nymph)

Fig. 9: Showing reduction in the egg fertility of female Dysdercus cingulatus emerged following the topical application and injection of different doses of Muristerone to the 4th and 5th instar nymphs respectively.

A = 4th instar nymphs..... Topical application

B = 4th instar nymphs..... Injection

C = 5th instar nymphs..... Topical application

D = 5th instar nymphs..... Injection

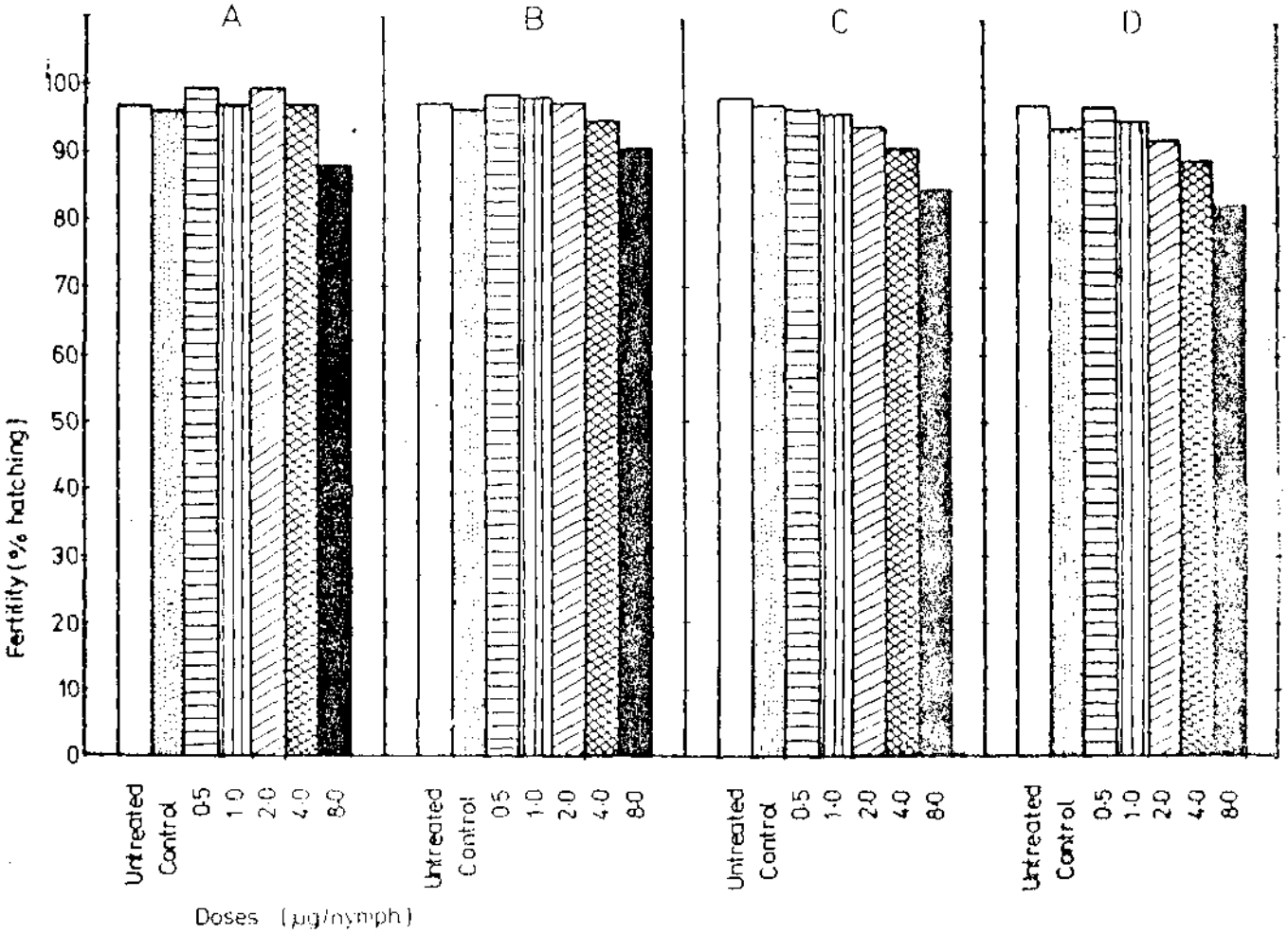

A
B
C
D
Fertility (% hatching)
100
90
80
70
60
50
40
30
20
10
0
Untreated Control
0·5
1·0
2·0
4·0
8·0
Doses (µg/nymph)

Fig. 10: Showing total nymphal mortality up to adult emergence in Fi generation following the topical application and injection of different doses of Muristerone to the 4th and 5th instar nymphs of Dysdercus cingulatus respectively in F generation.

A = 4th instar nymphs.....Topical application

B = 4th instar nymphs.....Injection

C = 5th instar nymphs..... Topical application

D = 5th instar nymphs.....Injection

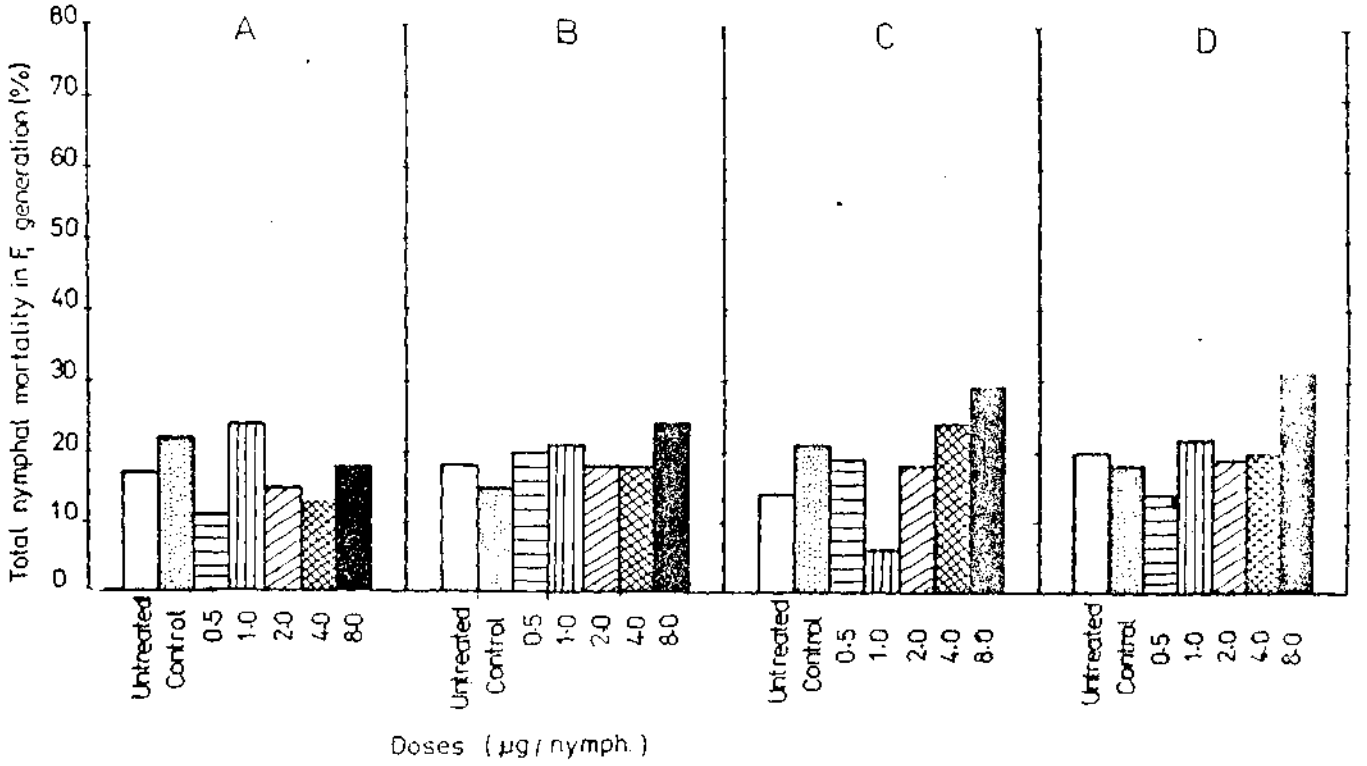

Total nymphal mortality in F1 generation (%)
0
10
20
30
40
50
60
70
80
A
B
C
D
Untreated
Control
0.5
1.0
2.0
4.0
8.0
Doses (µg / nymph.)

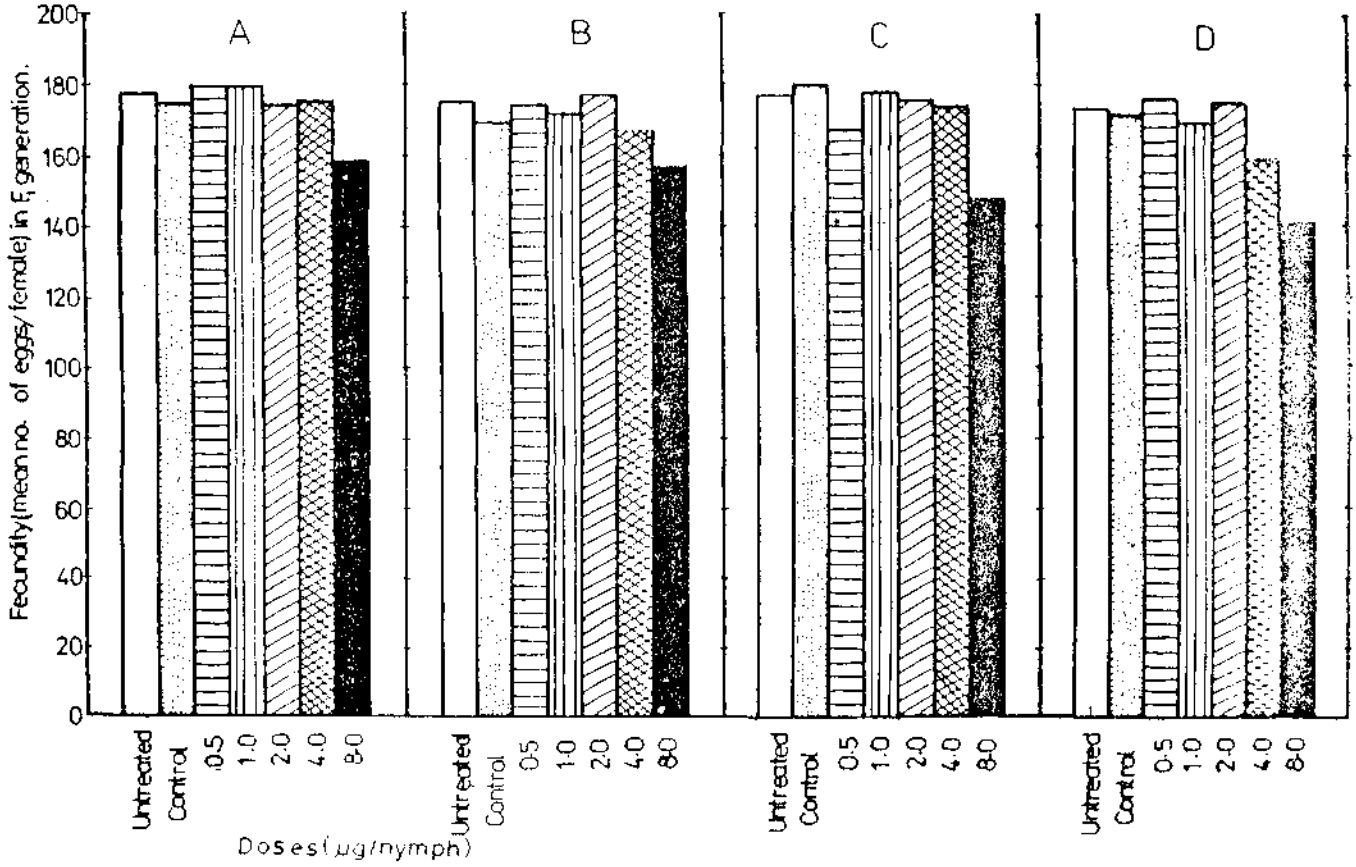
Fig. 11: Showing reducton in the fecundity of female Dysdercus cingulatus (F_1 generation) following the topical application and injection of different doses of Muristerone to the 4th and 5th instar nymphs respectively in F generation.

A = 4th instar nymphs.....Topical application

B = 4th instar nymphs.....Injection

C = 5th instar nymphs.....Topical application

D = 5th instar nymphs.....Injection


A
B
C
D
Fecundity(mean no. of eggs/female) in F1 generation.
200
180
160
140
120
100
80
60
40
20
0
Untreated Control
0·5
1·0
2·0
4·0
8·0
Doses(µg/nymph)

V. DISCUSSION

The effects of moulting hormones and their analogues including synthetic analogues as well as phytoecdysones on the growth and reproduction have been studied in several species of insects. The results reveal that there is a wide variation of response and susceptibility of different insects species against an ecdyson . Similarly, the response of a single species varies against different ecdysones. Exogenous ecdysones have been generally reported causing inhibition of larval and pupal development, moulting disorders, suppression of metamorphosis, inhibition of ovarian development and oogenesis as well as reduction in the fecundity and fertility. However, in certain species these hormones were found stimulating the growth and reproduction. Several explanations have been put forward to account for the effects of the exogenous ecdysones on larval and pupal development. Robbins et al. (1968 and 1970) on the basis of ingestion studies with several ecdysteroids suggested that the inhibition of larval development is related to the hormonal activity of these steroids. This conclusion was based on the observation that these effects could be related to moulting and morphogenesis and antigonadotropic activity. Kubo et al. (1981) showed

that oral administration of ecdysteroids caused ecdysis inhibition through successive moults until death occured and the effect was overtly hormonal rather than simple toxicity. Earlier, Mansingh (1976) on the basis of observations on Rhodnius prolixus suggested that the larval mortality could have been the result of some physiological derangement. Singh and Russell (1982) on the basis of their studies of dietary effects of a large number of ecdysteroids suggested that although there was no direct evidence that the effect of ingested ecdysteroids was related to their moulting hormone activity, however, the occurence of prothoeletic form (6%) in all of the 13 ecdysteroids treatmont except those of two piperonylate esters and the inference in normal puparium formation and adult emergence in some cases pointed to such a relationship.

In the present investigation on D. cingulatus application of 0.5 µg, 1.0 µg, 2.0 µg, 4.0 µg and 8.0 µg per 4th or 5th instar nymph by either topical or injection method caused nymphal mortality by all doses, which increased with the successive stronger doses. Total nymphal mortality was comparatively higher when the hormone was applied on the nymphs of 4th instar than that of the 5th instar. Further injection of the corresponding doses in even 5th instar was more effective than the topical application on mortality as well as other disorders.

Topical application and injection of the strongest dose (8.0 µg/nymph) of Muristerone to 4th instar nymphs of D. cingulatus caused the total nymphal mortality by 78% and 66% respectively whereas, on treating the 5th instar nymphs with the same dose the respective nymphal mortality was 41% and 48%. As reported by Ahmad and Khan (1985) Triol (an ecdyson analogue) was more lethal than Muristerone to this species. Injection of 4.0 µg Triol per nymph of 4th and 5th instar caused 65% and 43% nymphal mortality respectively (Ahmad and Khan, 1985). Whereas, in the present data, injection of the similar dose of Muristerone to the corresponding instars of this species could cause 48% and 32% nymphal mortality. On the other hand, α-ecdyson was even less fatal than Muristerone because injection of 4.0 µg/nymph α-ecdyson to the 4th and 5th instar nymphs of D. cingulatus caused only 21% nymphal mortality (Ahmad, 1983). On Hieroglyphus nigroreplectus (5th instar) injection of 6.0 µg/nymph β-ecdyson and Makisterone A (a phytoecdyson) respectively resulted in 14% and 38% nymphal mortality (Khan et al., 1984). Thus on this grasshopper these hormones (β-ecdyson and Makisteron A) are less fatal as compared to Muristerone on D. cingulatus. However, injection of 6.0 µg/larva of Makisterone A to the 5th and 6th instar larvae of

Spodoptera litura resulted in 32% and 20% loss in the larvae up to adult emergence. Singh and Russell (1982) in housefly recorded 84%, 77%, 63%, 25%, 10% and 9% larval mortality following the ingestion of 100 ppm 20-hydroxyecdyson (β-ecdyson), 5, 20-dihydroxyecdysone (analogue) and phytoecdysones Poststerone, Makisterone A, Ponasterone A and Dacrysterone respectively. Thus the phytoecdysones proved to be comparatively less fatal as compared to β-ecdysone (natural ecdyson). Earlier, Thompson (1970) reported that Ponasterone A, Cyasterone, (phytoecdysones) and Triol (analogue) added to the larval diet of housefly at 150 µg/g caused 80%, 22% and 100% inhibition of adult emergence. Besides these 12 more synthetic analogues labelled as compounds II, IV, V, VI, IX, X, XI, XII, XIV, XV, XVII and XVIII at the similar concentration caused inhibition in adult emergence ranging from 14 to 100%. More data further confirm that the analogues of natural ecdysones are much more effective in inhibiting the larval population of the insects than the phytoecdysones. On the other hand, confused beetle, Tribolium confusum larval diet containing 0.5% of β-ecdyson , Ponasterone A, Cyasterone, compounds I (Triol), IV, V, VII, VIII, IX, X and XIV respectively caused 28%, 100%, 16%, 100%, 100%, 70%, 29%, 12%, 69%, 40%, and 27% inhibition on larval development. Kubo et al.

(1983) reported that ingestion of 20-hydroxyecdyson (B-ecdyson) by the larvae of Bombyx mori resulted in death without moulting and death during the promoted moulting as well as inhibition of growth with and without effect on moulting . These effects were dependent upon the concentrations of the hormone, the precise developmental stage of the treated larvae and duration of exposure to the exogenous ecdysteroid.

Following the application of Muristerone (topical or injection) both on 4th and 5th instar nymphs of D. cingulatus the nymphal longevity was shortened only by 4.0 µg/nymph and 8.0 µg/nymph. The maximum reduction in the longevity was 24 to 32 hours in the 5th instar following the injection of 8.0 µg/nymph of the hormone. Whereas, the injection of similar dose to the 4th instar nymphs reduced longevity of this instar by 20 to 28 hours. Similarly, the topical application of this dose to the 5th and 4th instar nymphs resulted in shortening their duration by 24 to 30 hours and 20-28 hours respectively. Apparently, there is little difference in the reduction of longevity by injection and topical application of a dose. When the 4th instar nymphs injected with 8.0 µg Muristerone/nymph moulted to 5th instar the longevity of the 5th instar was shortened by 16 to 24 hours. However, lower doses were

ineffective on nymphal longevity. The longevity of 5th instar nymphs of D. cingulatus following the injection of 4.0 µg Triol per nymph was reduced by 16-24 hours (Ahmad and Khan, 1987) which was almost the same (16-20 hours) by similar dose of Muristerone as presently observed. Reduction in the larval longevity was also reported by β-ecdyson in Spodoptera litura. According to Ahmad and Khan (1982) ingestion of 6.0 µg β-ecdyson per larva of 5th and 6th instar of this species reduced their longevity by 10-15 hours and 24-36 hours respectively. But following moulting to the next instar (5th instar) the longevity was less affected and with respect to 4.0 µg and 6.0 µg β-ecdyson these were reduced by 8-10 hours and 15-20 hours respectively. They concluded that probably β-ecdyson was neither digested nor hydrolysed in the digestive tract, but it was absorbed by the midgut and transported to the haemolymph thereby enhanced the titre of the naturally circulating hormone which accelerated the moulting process.

Application of higher concentration or doses of ecdysteroids has been found causing the production of supernumerary instar as well as larval, pupal and adult malformation in various insect species. Ahmad and Khan (1982)

observed the production of 5% supernumerary larvae following the ingestion of 6.0 µg β-ecdyson by the 5th and 6th instars of Spodoptera litura. Further, in Diacrisia obliqua malformation at larval, pupal and adult stages were seen following the ingestion of sublethal doses of α-ecdyson and Triol (Ahmad, 1983). Kobayashi and Burdette (1962) in B. mori found formation of pupal-adult mixtures following the injection of ecdysterone (β-ecdyson) or Inokosterone (a phytoecdyson) at 10.0 µg/pupa. In the same species dose dependent effect on early adult emergence and formation of pupal and adult mixtures were also observed by Kambysalis (1967) following the injection of Inokosterone (0.1 - 10.0 µg/pupa). Further, Giebultowicz et al. (1980) observed that injection of 20-hydroxyecdyson (β-ecdyson) into later stage last inster larvae of Ephestia kuhniella resulted in the formation of larval pupal intermediates. In the present experiments topical application and injection of Muristerone to 4th and 5th instar nymphs of D. cingulatus also caused malformation in both nymphs and adults. Production of supernumerary nymphs occured following the application of only higher doses (4.0 µg and 8.0 µg) whereas, adult malformation was caused by all the doses tested. Injection of 8.0 µg Muristerone per nymph of 4th instar led to the production of 4% supernumerary nymphs. Whereas, application of the similar

by both methods to the 5th instar nymphs resulted in the formation of 16% and 11% supernumerary nymphs respectively. Thus application of this phytoecdyson at the most advanced stage of larval growth proved to be much disturbing. Further, in this species maximum percentage (57.69) of malformation was observed in adults emerged from 5th instar nymphs injected with 8.0 µg Muristerone whereas the similar dose injected into 4th instar nymphs resulted in the emergence of 47.06% malformed adults. Similarly, topical application of this dose on the 5th and 4th instar nymphs caused emergence of respectively 62.54% and 47.62% malformed adults . It was therefore, further confirmed that application of this hormone at fully advanced stage of larval growth is most effective in disturbing growth and normal structures.

Both fecundity and fertility of D. cingulatus were adversely affected by the exogenous Muristerone. Although reduction in the fecundity of the females was dose based, the fertility of the eggs was only affected by stronger doses. Further, the effect on both fecundity and fertility was more prominent when the application was made on the 5th instar nymph than on those of the 4th instar. Topical application as well as injection of 8.0 µg Muristeron/nymph to the 4th instar nymphs caused reduction in the fecundity of the

emerged females by 45.89% and 51.08% respectively. Whereas, there was significant effect on the fertility of the eggs laid by the affected females. By similar application on 5th instar nymphs the respective fall in the fecundity was 58.16% and 62.74% and that of the fertility was 12.83% and 11.7%. Jalaja et al. (1976) found 50% fall in the fecundity of D. cingulatus following injection of 4.0 µg β-ecdyson/female (one day old) and further application of the same dose on three consecutive days (total of 16.0 µg/female). In the same species injection of 4.0 µg Triol (an analogue) per nymph of 4th and 5th instar in a single dose respectively resulted in 17% and 21% reduction in the fecundity of the emerged females whereas, the respective decrease in the fertility of the eggs was 43% and 53% (Ahmad and Khan, 1985). Thus in comparison to β-ecdyson as tested by Jalaja et al. on D. cingulatus females, 'Triol' (an analogue) is comparatively and significantly effective as reproductive inhibitor of this species even if applied in the advanced larval stage by Ahmad and Khan (1985).

In the present experiments Muristerone injected into 5th instar nymphs of D. cingulatus even at 4.0 µg/nymph inhibited the fecundity of the emerged females by 45%. But unlike Triol, even 8.0 µg/nymph Muristerone had

negligible effect on the fertility of the eggs. Similar dose (4.0 µg/nymph) of Triol injected to 4th and 5th instar nymphs caused 43% and 53% reduction in the egg fertility. On the other hand, ∝-ecdyson was less effective than Triol (Ahmad, 1983). The injection of 4.0 µg ∝-ecdyson/nymph to 4th and 5th instar of D. cingulatus reduced the fecundity by only 3.3% and 25.2% respectively (Ahmad, 1983). However, corresponding Muristerone doses caused greated reduction in the fertility of the eggs of affected females of D. cingulatus as observed in the present experiments.

In Spodoptera litura ingestion of β-ecdyson (6.0 µg/larva) by the 5th and 6th instar larvae reduced the fecundity of the emerged females by 61.54% and 89.95% respectively whereas, egg fertility of such females dropped by 32.96% and 66.33% respectively. On the other hand, Makisterone A (a phytoecdyson) was potent inhibitor of fecundity but had no effect on the egg fertility. Injection of 6.0 µg Makisterone A to the 4th and 5th instar nymphs reduced the fecundity of the affected females by 12.50% and 13.52%. But egg fertility was similar to that of the control (Khan et al., 1994). According to Thompson (1970) Ponasterone A (a phytoecdyson) was not a reproduction inhibition for Tribolium

confusum whereas, Triol inhibited by more than 90% at 1.0% concentration given through larval diet and there was no recovery on the inhibitive effect even at the end of 8th week after the treatment. But Thompson (1970) further reported that dietary α-ecdyson, 20-hydroxyecdyson, Ponasterone A, Inokosterone, cyasterone and Triol caused ovarian inhibition by 100%, 46%, 80%, 56%, 96% and 100% respectively.

So far there was no information on the residual effect of the ecdysones in the larval stages and adults of the following generations. In the present experiments on D. elaqui.tus the residual effect of the exogenous ecdyson (Muristerone) was followed in F_1 and F_2 generations also. It was found that there was significant loss in adult emergence (in F_1 generation) following the application of the strongest dose (8.0 µg/nymph) on the 5th instar nymphs of P generation. Lower doses did not show such an effect. The topical application as well as injection of 8.0 µg Muristerone per 5th instar nymph of P generation resulted in the death of nymphs of F_1 generation by 29% and 32% respectively. Such change was not seen when 4th instar nymphs of P generation were treated similarly. This observation suggested that ecdyson application at the level of 8.0 µg to the 5th instar nymphs of D. elongatus might produce residual effect in the

next generations as well as though less marked in F_2 generation. Similarly, the administered higher doses (4.0 µg and 8.0 µg) of this phytoecdysone to both 4th and 5th instar nymphs of F generation reduced the fecundity of the females of F_1 generation but egg fertility was unaffected. Further, the drop in the fecundity was more prominent in case of the females emerged from 5th instar treated nymphs of F generation than that of the 4th instar. Topical application and injection of 8.0 µg/nymph Muristerone to the 4th instar nymphs of F generation caused fall in the fecundity of the emerged females of F_1 generation by 9.33% and 7.49% respectively. Whereas, respective fall in fecundity of the females of F_1 generation emerged from 5th instar treated nymphs (F generation) were 16.04% and 17.91%. This again confirmed stronger residual effect of this ecdysone when applied on the fully grown nymphs than the earlier stage. In F_2 generation there was normal growth and reproduction like that of the controls.

On the basis of present data on D. cingulatus it can be concluded that exogenous Muristerone in its sublethal doses can cause significant mortality, disorders in moulting, metamorphosis as well as reduction in the fecundity and fertility within the same generation. Further, the residual effect

on mortality and fecundity continued in the subsequent (F_1) generation of this species. Thus Muristerone, an ecdyson of plant origin is one of the potent hormones in bringing out check on its population.

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